

**“SYNTHESIS, CHARACTERIZATION AND *INVITRO*  
ANTI INFLAMMATORY AND ANTHELMINTIC  
ACTIVITIES OF 1,3,4-OXADIAZOLE DERIVATIVES”**

*A dissertation submitted to*

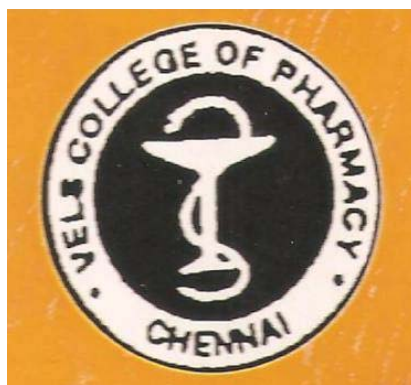
**THE TAMIL NADU Dr.M.G.R. MEDICAL UNIVERSITY  
CHENNAI - 600032.**

*In partial fulfillment of the requirements  
for the award of degree of*

**MASTER OF PHARMACY  
IN  
PHARMACEUTICAL CHEMISTRY**

*Submitted by*  
**D.BHARATHI, B.Pharm.,**

*Under the guidance of*  
**Asst. Prof. Dr. S. HEMALATHA, M.Pharm, Ph.D**



**MARCH 2010**

**DEPARTMENT OF PHARMACEUTICAL CHEMISTRY  
VEL'S COLLEGE OF PHARMACY  
OLD PALLAVARAM, CHENNAI-600117.**

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*DEDICATED TO  
MY BELOVED  
PARENTS*

# *ACKNOWLEDGEMENT*

## ACKNOWLEDGEMENT

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# **CONTENTS**

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# *CHAPTER-I*

## *INTRODUCTION*

## 1. INTRODUCTION<sup>1</sup>

Medicinal or Pharmaceutical chemistry is a scientific discipline at the intersection of chemistry and pharmacology involved with designing, synthesizing and developing pharmaceutical drugs. Medicinal chemistry is concerned with the organic, analytical and biological aspects of new drugs and involves the identification, synthesis and development of new chemical entities suitable for therapeutic use. It also includes the study of existing drugs, their biological properties and their Quantitative structure activity relationship. Pharmaceutical chemistry is focused on quality aspects of medicine and aims to assure fitness for the purpose of medicinal products.

In the glorious days of the 1950's and 1960s chemists envisioned chemistry as the solution to a host of society's needs. Indeed, they created many things which improve the quality of life on earth like dyes, plastics, cosmetics and other materials. At the same time chemistry brought about medicinal revolution that is, through antibiotics which conquered infectious disease. All these things prove Do Pont slogan **“Better things for better living through chemistry”**.

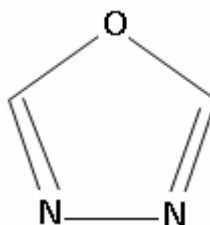
Before the development of chemistry as a science, drugs that had been used were natural organic products or inorganic material, herbal and pharmacopoeias listed plants that had been in use for thousands of years and resulted from efforts by various societies for the various illness and diseases encountered and recognized.

The science now employs the most sophisticated development in technology and instrumentation including powerful molecular graphics with drug design software. Significant advances in x-ray crystallography and NMR have made the molecule to obtain detail representation of enzymes in other drug receptor. Thus the medicinal chemistry occupies a strategic position at the interface of biology and chemistry and it has enormous growth.

Among the wide variety of heterocyclic that have been explored for developing pharmaceutically important molecules. 1, 3, 4 –oxadiazole derivatives have played a vital role in the medicinal chemistry. There are large numbers of synthetic compounds with oxadiazole nucleus used for antibacterial, analgesic and anti-inflammatory activity. The

broad spectrum of biological activity of 1,3,4 oxadiazole derivatives prompted us to synthesize the compounds and screen them for pharmacological activities.

## NUCLEUS INTRODUCTION (1,3,4-OXADIAZOLE)



Molecular formula : C<sub>2</sub>H<sub>2</sub>N<sub>2</sub>O

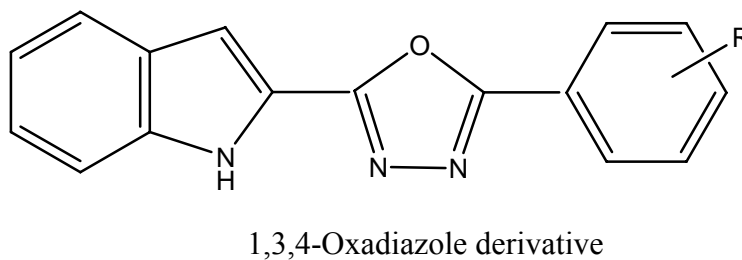
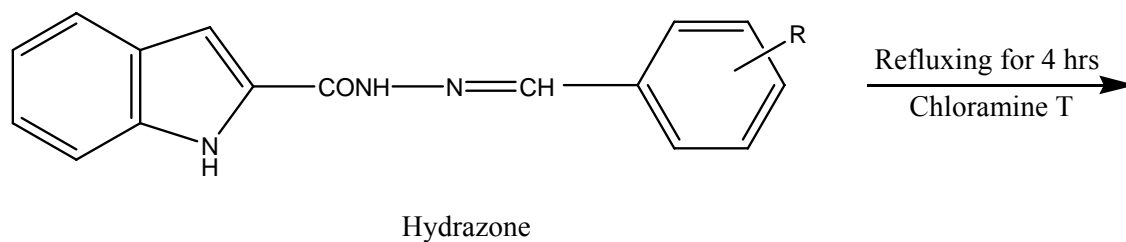
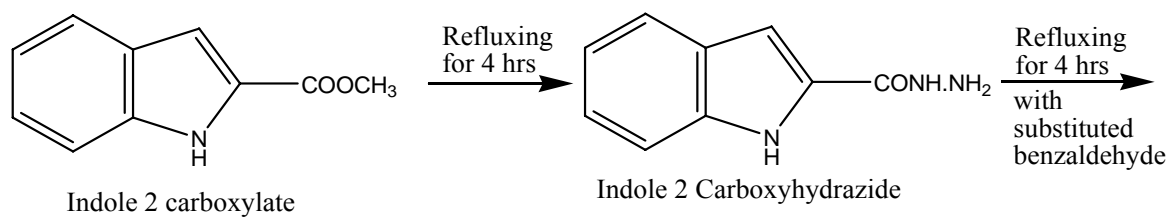
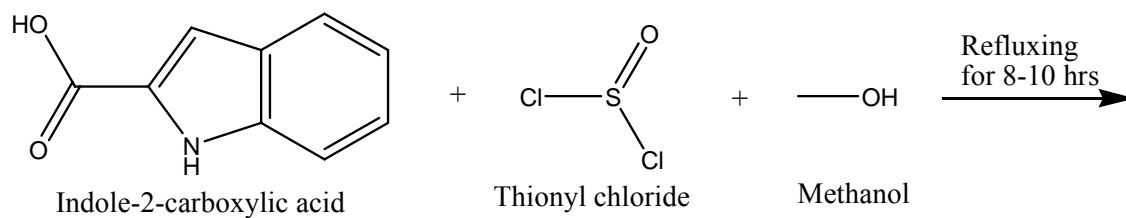
Molecular weight : 70.05g

Appearance : Liquid

Boiling point : 150-152<sup>0</sup>C

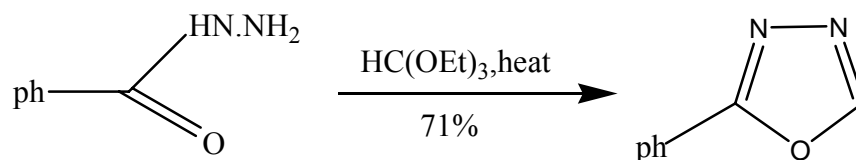
1,3,4 oxadiazoles are the heterocycles that have received considerable attention during last two decades as the potential antibacterial, fungicidal, insecticidal, herbicidal, anti inflammatory, analgesic, antipyretic, anti tubercular, sedative, hypnotics, hypoglycemic agents, dyes and x-ray contrast materials .

Derivatives of 1,3,4-oxadiazole constitute an important family of heterocyclic compounds. There are several methods available in the literature for the synthesis of 1,3,4-oxadiazole. However, some of these methods suffer from disadvantages such as long reaction times, lower yields; require severe condition by using strong or toxic oxidants. The oxadiazole chemistry has been developed extensively and is still developing. Presently, there are a number of drugs used clinically, which comprise oxadiazole moiety in association with various heterocyclic rings. In view of this a project was undertaken to synthesize a new series of 1,3,4-oxadiazoles containing the aldehyde. For these reasons the chemistry of 1,3,4-oxadiazoles has been the subject of many investigations.

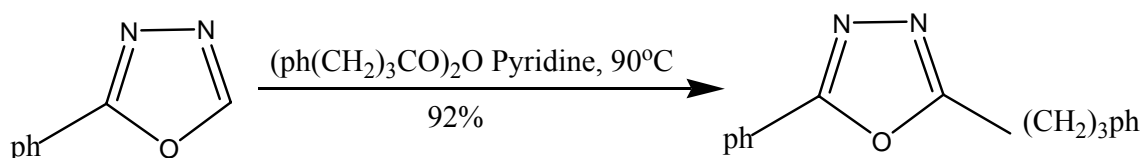
SYNTHESIS OF 1,3,4 OXADIAZOLE<sup>43</sup>

## RING SYNTHESIS

1,3,4-oxadiazoles are available by cyclodehydration of  $N,N^1$ -diacylhydrazines or their equivalents. They are also available from tetrazoles or by oxidative cyclisation of acyl hydrazones.

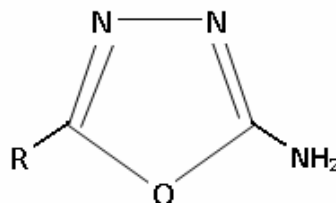


1,3,4-oxadiazoles are formed on heating tetrazoles with acylating agents via rearrangement of a first formed 2-acyl derivatives.



## LEAD MOEITY

As the oxadiazole and aldehyde with amino group possess diverse biological activity, the present work has been done on constructing the two moieties and synthesizes compounds which were screened for their anti inflammatory activity.



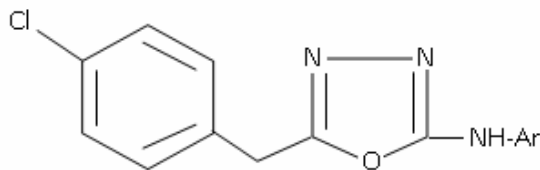
$\text{R}$  - Different Aldehydes

# CHAPTER-II

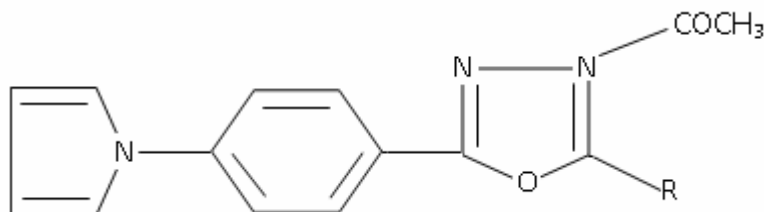
## REVIEW OF LITERATURE

## 2. REVIEW OF LITERATURE

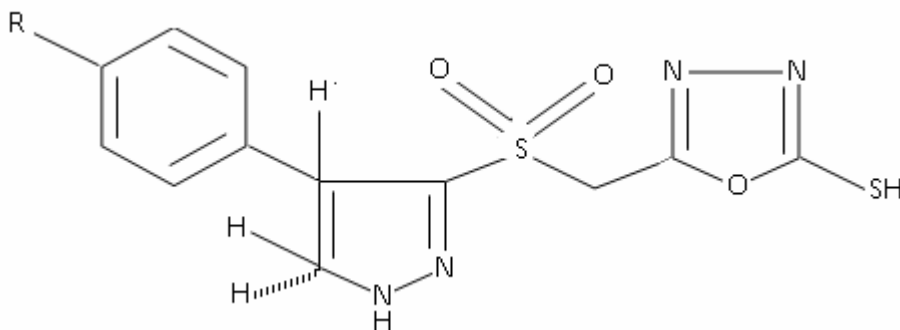
- **Praveen Kumar et al. (2010)<sup>12</sup>** synthesized 1-[(5 substituted-1,3,4-oxadiazol-2-yl) methyl]-4-propylpiperazines was carried out by refluxing the 1-propyl piperazine with ethylchloroacetate in dry acetone in presence of potassium carbonate and subsequent hydrazinolysis with hydrazine hydrate. Finally 2-(4-propylpiperazin-1-yl)aceto hydrazide was treated with appropriate carboxylic acids in presence of phosphorous oxy chloride to produce title compounds. The newly synthesized compounds were tested for its anti bacterial, anti fungal and anthelmintic activity.
- **Ghodsi et al. (2009)<sup>13</sup>** synthesized 2-amino-1, 3, 4-oxadiazole compound via cyclization of 2-(2,4-dichlorothiazol-5-yl)methylene)hydrazine carboxamide in the presence of bromine. Diazotation of compound 2-amino 1, 3, 4 oxadiazole in hydrochloric acid in the presence of copper powder results 2 chloro 5(2, 4-dichlorothiazol-5-yl) 1, 3, 4 oxadiazole in which aminogroup was substituted to chloro. In this they have done only characterization and elemental analysis.
- **Fuloria et al. (2009)<sup>14</sup>** synthesized new 1-(2-aryl-5-phenethyl-1, 3, 4 oxadiazol-3(2H)-yl) ethanones by cyclization of imines using acetic anhydride. The products were evaluated for antibacterial and antifungal activity. Among the newly synthesized compounds, 1-(2-(4-(dimethyl amino)phenyl)-5-phenethyl-1,3,4-oxadiazol-3(2H)-yl)ethanone and 1-(2-(4-chloro phenyl)-5-phenethyl-1,3,4-oxadiazol-3(2H)-yl)ethanone were found to possess maximum activity against the tested strains of *S. aureus* and *P. aeruginosa*
- **Husain et al. (2009)<sup>15</sup>** synthesized 1,3,4 oxadiazoles with eroyl propionic acid by condensation reaction and evaluated anti inflammatory, analgesic and antibacterial activity.
- **Desai NC et al. (2008)<sup>16</sup>** synthesized thiosemicarbacides, 1,2,4 triazoles, 1,3,4-oxadiazoles derivatives by using alkaline ethanolic solution of iodine containing potassium iodide, orthophosphoric acid and aqueous potassium hydroxide solution. These compounds have been assayed for their anti microbial activity against gram positive and gram negative micro organism.



- **Joshi SD et al. (2008)<sup>17</sup>** synthesized new 4-pyrrol-1-yl benzoic acid hydrazide analogs and some derived oxadiazole, triazole and pyrrole ring system. A novel class of potential antibacterial and anti tubercular agent.

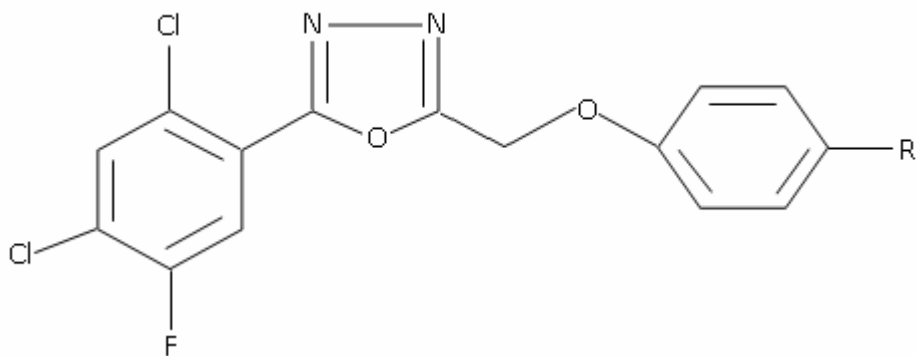


- **Padmavathi V et al. (2008)<sup>18</sup>** synthesized novel sulfone- linked bis heterocycles. The compounds on condensation with 2-carboxy hydrazide and different aromatic aldehydes afford the corresponding compound and screened for antimicrobial activity.



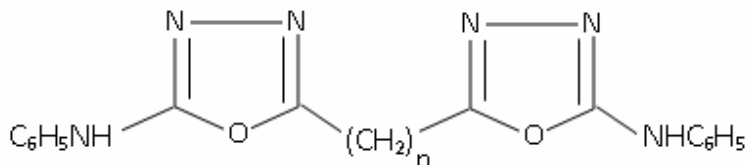
- **Marisithambaram Karthikeyan et al. (2008)<sup>19</sup>** synthesized 2,4-dichloro-5-fluoro phenyl containing oxadiazoles have been synthesized by mannich reaction of 5-(5-methylisoxazol-3-yl)-1,3,4-oxadiazol-2thiol with amines and cyclization of hydrazones with acetic anhydride. The compounds synthesize have been confirmed by their elemental analysis and spectral data. The antimicrobial activities have also been evaluated.





- **Shakya et al. (2008)<sup>20</sup>** Synthesis and biological evaluation of Schiff base of 2-Amino-5-(2-Chlorophenyl)-1,3,4-oxadiazole using aromatic aldehydes. The chemical structure of these compounds was confirmed by elemental analysis, IR, <sup>13</sup>C NMR. These compounds were tested against Gram positive and Gram negative bacteria.

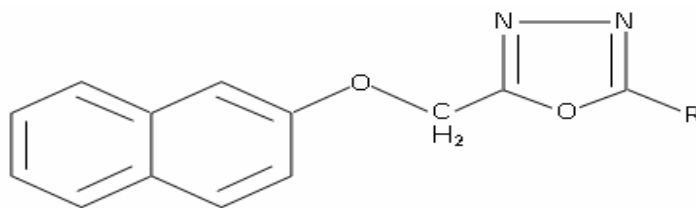
- **Ajay K. Behera et al. (2006)<sup>22</sup>** synthesized bis (thiadiazolyl/ oxadiazolyl/ triazolyl) alkanes from dicarboxylic acid and screened for anti fungal activity



- **Ravindra et al. (2006)<sup>23</sup>** reported the condensation of naphtha (2, 1-b) furan-2-carboxyhydroxide with different aromatic aldehydes affords the corresponding N-[(IE)-aryl methylene]-naphtho[2,1-b] furan 2-carboxyhydroxide. These undergo cyclization with acetic anhydride and mercuric oxide to yield 3-acetyl-5-naphtho[2-2-b] furan-2-yl-2-aryl-2,3 dihydro-1,3,4 oxadiazoles and 2 naphtho[2-1-b] furan 2-yl-5-aryl 1,3,4-oxadiazoles on refluxing with carbon disulphide and ethanolic potassium hydroxide followed by acidification. These have shown antimicrobial, anti inflammatory activity.
- **Naga et al. (2006)<sup>24</sup>** synthesized 2,5 disubstituted 1,3,4 oxadiazole by condensation of 4-methoxy benzohydrazide with different aromatic acids in presence of phosphoryl chloride. The structural assignment of this compound has been made on

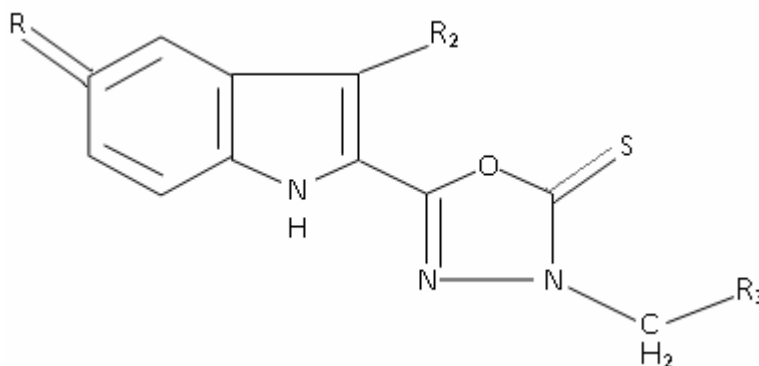
the basis of elemental analysis, UV, IR,  $^1\text{H}$ NMR and different strains of bacteria and fungi which was compared with that of standard antibiotics such as chloramphenicol and griseofulvin (50  $\mu\text{g/ml}$ )

- **Khan MSY et al. (2006)**<sup>25</sup> synthesized some new 1, 3, 4-oxadiazole derivatives with different aromatic acids and aromatic aldehydes by cyclization –oxidation reaction of acyl hydrazones. All the products have been evaluated for their anticonvulsant and antibacterial activity.

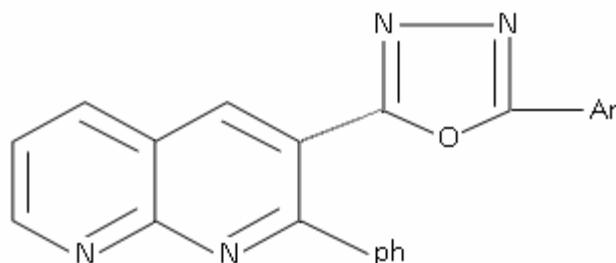


- **Mohan TP et al. (2004)**<sup>26</sup> Condensation of (2-oxo-3-phenyl-2H-(1,8)naphthyridin-1-yl)-acetic acid hydrazide with different acetophenones yields the corresponding acetophenone (2-oxo-3-phenyl-2H-(1,8)naphthyridin-1-yl)methyl carboxy hydrazones which on treatment with acetic anhydride affords the respective 1,3,4-oxadiazole derivative which have been confirmed on the basis of analytical and spectral data.
- **Holla et al. (2004)**<sup>27</sup> synthesized number of 1,3,4-oxadiazoles 2-chloropyridine is prepared starting from easily available 2-chloro-5-chloromethylpyridine via 2-chloro pyridine-5-acetic acid hydrazide. On reacting with aryl chloride in the presence of  $\text{POCl}_3$  yields 2-chloro-5-(aryl 1,3,4-oxadiazol-2-yl)methyl pyridine by esterification process and evaluated insecticidal activity.
- **Khan et al. (2004)**<sup>28</sup> synthesized 1,3,4-oxadiazole and isoniazid moieties are important because of their versatile biological actions. In present studies the oxadiazole has been built with isoniazid moiety and a few compounds in this series have been synthesized, evaluated their anti-inflammatory and biological properties.

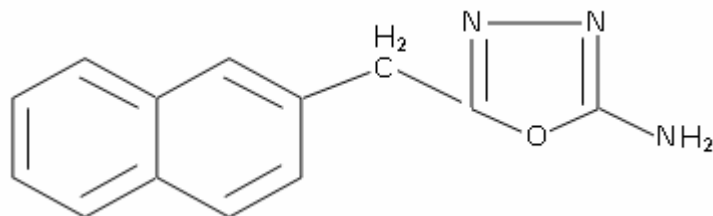
- **Bhat et al. (2004)<sup>29</sup>** synthesized a number of 1,3,4 oxadiazole derivatives in which 2,4 dichloro -5- fluoro benzoyl hydrazine on reacting with aromatic acids in presence of phosphorus oxychloride affords 2,5 disubstituted 1,3,4 oxadiazoles and evaluated potential antibacterial and anticancer agents.
- **Aydogan et al. (2002)<sup>30</sup>** reported on synthesis and electronic structure of new aryl and alkyl-substituted 1,3,4-oxadiazole-2-thione derivatives by the ring closure reaction of various acyl hydrazides with carbon disulphide. Mannich bases for some of these compounds were also synthesized by condensation with benzaldehyde and primary amines. All new compounds were characterized by spectral data. Most of them were tested for their anti bacterial and anti tubercular activity.
- **Hui et al.(2002)<sup>31</sup>** reported 5-(5-methylisoxazol-3-yl)-4-substituted aminomethyl -2-thio-1,3,4-oxadiazoles and 4 acetyl-2-(5-methylisoxazol 3-yl)-5-substituted-1,3,4-oxadiazole have been synthesized by mannich reaction of 5-(5-methyl isoxazol-3-yl) 1,3,4-oxadiazol-2-thiol with amine and cyclization of hydrazones with acetic anhydride respectively and evaluated antibacterial activities.
- **Sreenivasulu N et al. (2001)<sup>32</sup>** 2-(Benzimidazol-2-yl)benzoyl hydrazide when condensed with aromatic acids in the presence of  $\text{POCl}_3$  afforded 2-aryl-5-(2-benzimidazol-2-yl)phenyl-1,3,4-oxadiazoles. The acid hydrazide on cyclisation with cyanogenbromide yield 2-amino-5-(2-(benzimidazol-2-yl-phenyl)-1,3,4oxadiazole. All the products have been evaluated for anti inflammatory and antimicrobial activity.



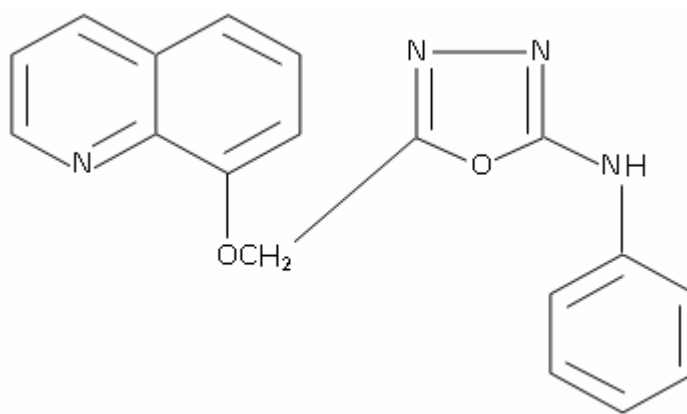
- **Mogilaiah k et al. (2001)**<sup>33</sup> synthesized pyrazole and 1,3,4 oxadiazole derivatives of 2-phenyl-1,8-naphthyridine with different aromatic aldehydes affords the corresponding N1-(1E)-aryl methylene)-naphtho (2,1-b) pyridine 2- carboxy hydrazide. These compounds undergo cyclization with acetic anhydride and mercuric oxide to yield 3-acetyl-5-naphthol(2,1-b)pyridine-2-yl-2aryl-2,3dihydro-1,3,4oxadiazole and refluxing with carbondisulphide and ethanolic potassium hydroxide. Followed by acidification with hydrochloric acid furnishes the above compound.



- **Kagthara et al. (1999)**<sup>34</sup> reported 2-(Benzimidazol-2-yl) benzoyl hydrazide when condensed with aromatic acids in the presence of  $\text{POCl}_3$  afforded 2-aryl-5-[2-benzimidazol-2-yl) phenyl]-1,3,4 oxadiazoles. The acid hydrazide on cyclisation with  $\text{CNBr}$  yield 2 amino-5-[2-(benzimidazol-2-yl phenyl)-1,3,4-oxadiazole which on reaction with aryl sulphonyl chlorides and substituted benzoyl chloride give the corresponding sulphonamides and amide respectively. All the products have been evaluated *in vitro* for their antimicrobial activity against several microbes and anti tubercular agent against mycobacterium tuberculosis.
- **Mohd.Amir et al. (1998)**<sup>35</sup> synthesized naphthyl methyl oxadiazoles, thiadiazole and triazole was obtained from the reaction of 2,4-dichloro thiazole-5-carboxaldehyde and semicarbazide . 2-Amino-1,3,4-oxadiazole compound was synthesized via the cyclisation of compound in presence of bromine .Diazotization of compound in hydrochloric acid in presence of copper powder results the compound.

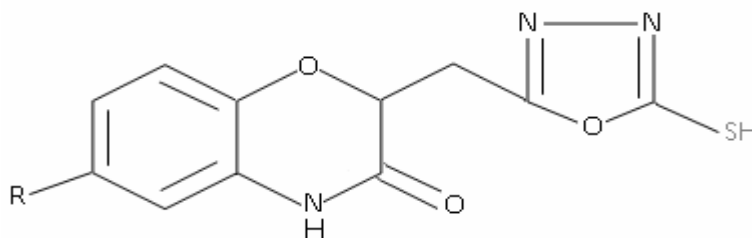


- **Mohd Amir et al. (1998)<sup>36</sup>** synthesized 5-(8-quinolinomethyl)-1,3,4-oxadiazole/1,3,4-thiadiazole and 1,2,4 (H) triazole screened for anti inflammatory activity.



- **Shah et al (1997)<sup>37</sup>** synthesized 2-aryl amino-5-p-(nicotinamido phenyl)-1,3,4-oxadiazoles starting from ethylnicotinamido benzoate which in turn obtained treatment of nicotinic acid with thionyl chloride by reacting with ethyl-para aminobenzoate in iodine. It has evaluated anti microbial agents.

- **Reddy VM et al. (1996)<sup>38</sup>** synthesized 2-[(3,4-Dihydro-3-oxo-2H-1,4-Benzoxazin - 2-yl)methyl]-5-(alkyl/arylthio)-1,3,4 oxadiazoles and screened for antimicrobial activity.



- **Ladva et al. (1996)**<sup>39</sup> reported the synthesis and biological activities (anticonvulsant, anti-inflammatory and antimicrobial) of a number of 2,5-disubstituted 1,3,4-oxadiazoles are described. 2-(4-isobutyl phenyl) propionic acid hydrazide is condensed with aromatic acids to yield 2-aryl-5-(2-methyl-4-isobutyl benzyl)-1,3,4-oxadiazoles.
  
  - **Kataky et al. (1990)**<sup>40</sup> reported the reaction of 2,4-di-chlorobenzoyl hydrazine and ethyl chloro formate and subsequent cyclisation in the presence of phosphorous oxy chloride will give 2-(2,4-di chlorophenyl)-1,3,4 oxadiazol-5-one(1) 4-N-Acyl aroyl-2-(2,4-dichlorophenyl)-1,3,4-oxadiazol-5-ones were prepared by the action of various aliphatic and aromatic acid chlorides on (1). The fungi toxic activity was reported.
-



# *CHAPTER-III*

## *RESEARCH ENVISAGED*



### 3.1 OBJECTIVE OF WORK

In modern era, the field of pharmaceutical chemistry is changing. Now most of the scientists of chemistry field are trying to prepare new synthetic analogs by simple or more involved modifications of the structures of the natural drugs, or by pure synthesis. By changes in structures of the natural drugs and following the leads, there have been got, for instance, many new agents with higher activity and concentrate more on molecular modeling and computer assisted drug design. The aim of the project work is to synthesize new heterocyclic derivatives with potential biological activity and less adverse effects.

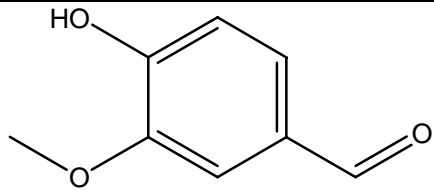
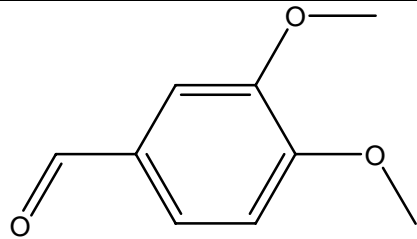
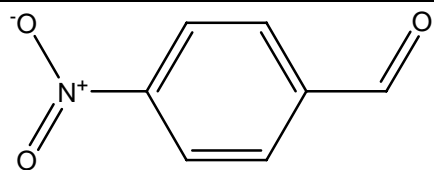
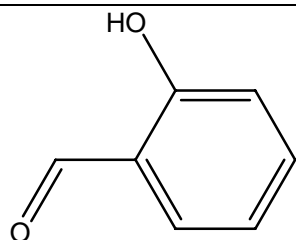
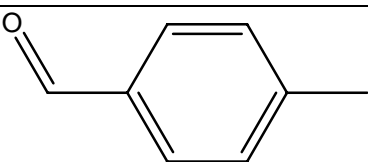
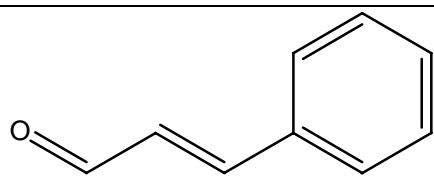
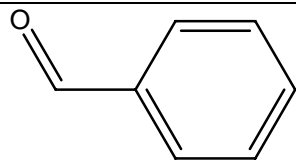
As from the literature oxadiazole derivatives are reported to have following varieties of activities:-

- Antifungal<sup>13</sup>
- Antibacterial<sup>16, 27, 31</sup>
- Insecticidal<sup>24, 25</sup>
- Anti inflammatory<sup>26,30,33,34</sup>
- Analgesic<sup>14</sup>
- Herbicide<sup>28</sup>
- Anti malarial<sup>33</sup>
- Anti convulsant<sup>23</sup>
- Anthelmintic Activity<sup>12</sup>
- Anti tumoral<sup>27</sup>
- Anti viral<sup>32</sup>
- Anti microbial<sup>36, 39</sup>
- Muscle Relaxant<sup>27</sup>
- Tranquilizer<sup>27</sup>
- Anti tubercular Activity<sup>32</sup>

Therefore based on the previously reported information concerning oxadiazole with different aldehyde it was planned to synthesize novel 1,3,4 oxadiazole with more biological and chemotherapeutic efficacy as compared to some previously reported 1,3,4 oxadiazole derivatives and with good yield.

So an attempt was made to synthesize these derivatives and to screen anti-inflammatory and anthelmintic activities.

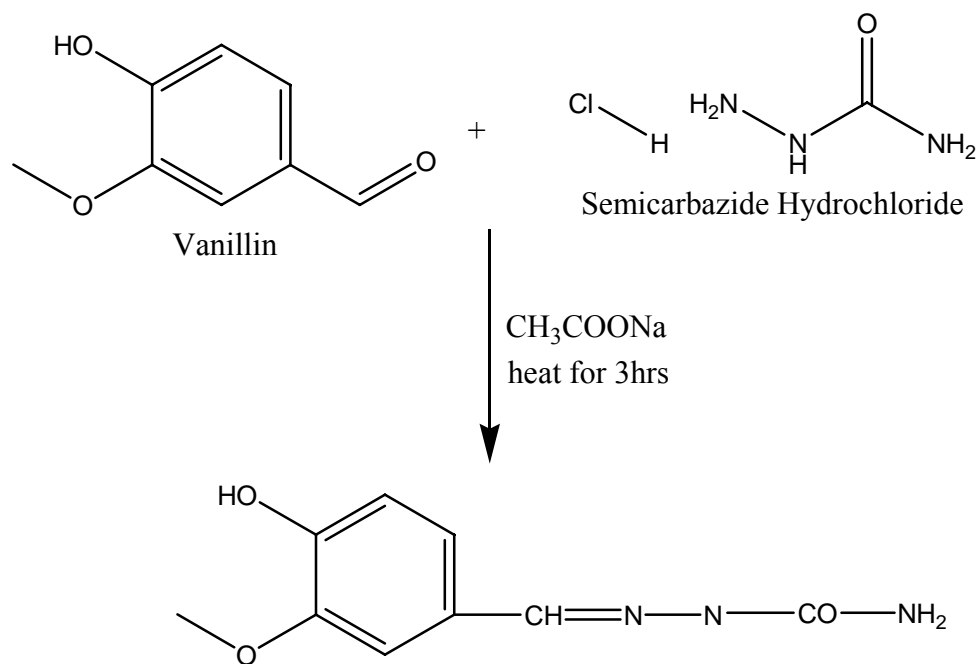
Table I - List of Aldehydes used in the Synthesis

S.NO	R	COMPOUND NAME
1		Vanillin
2		Veratraldehyde
3		p-nitrobenzaldehyde
4		Salicaldehyde
5		4-methylbenzaldehyde
6		Cinnamaldehyde
7		Benzaldehyde

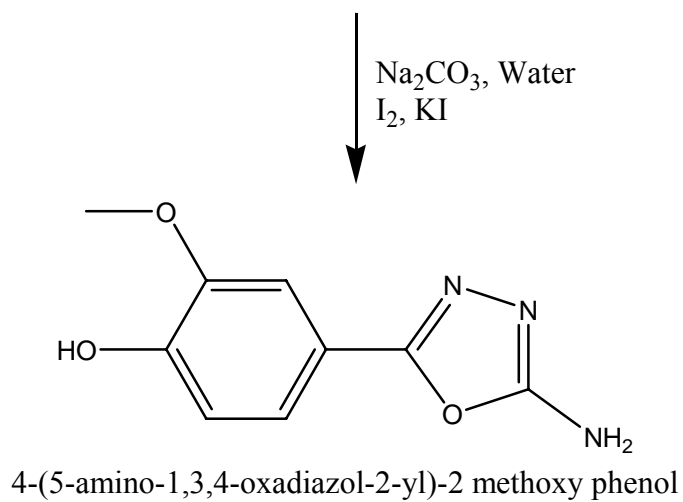
## 3.2 SCHEME

## SCHEME-I

## STEP-I

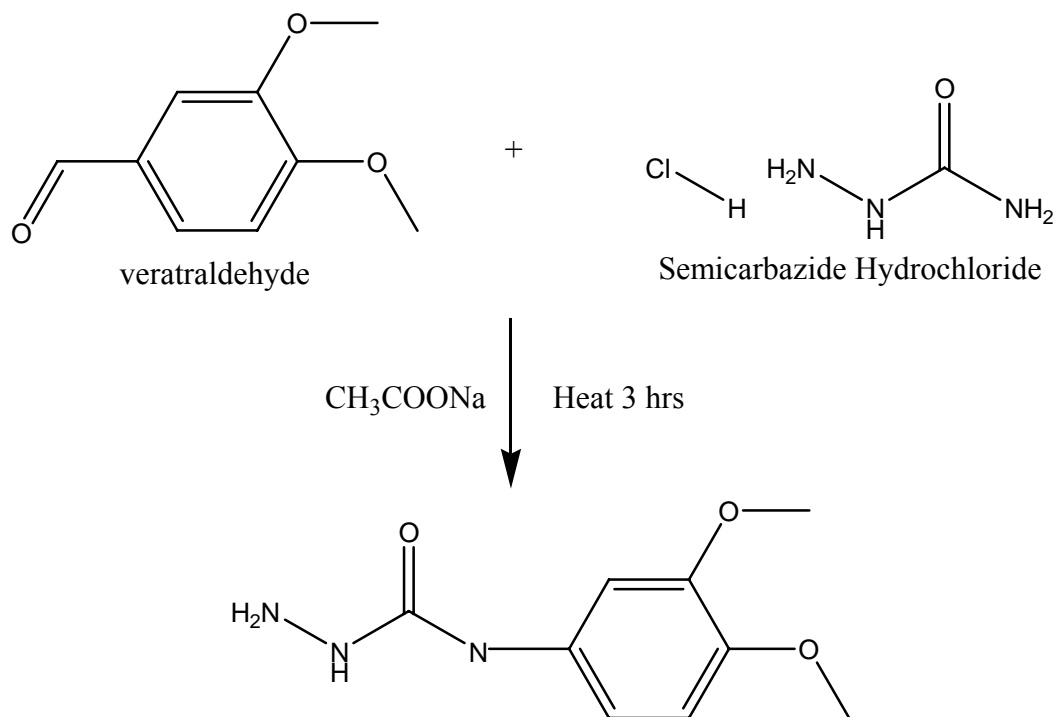


## STEP-II

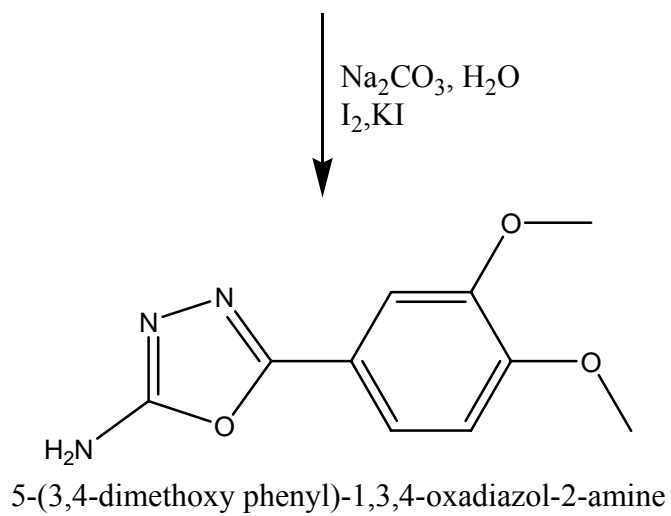


## SCHEME-II

## STEP-I

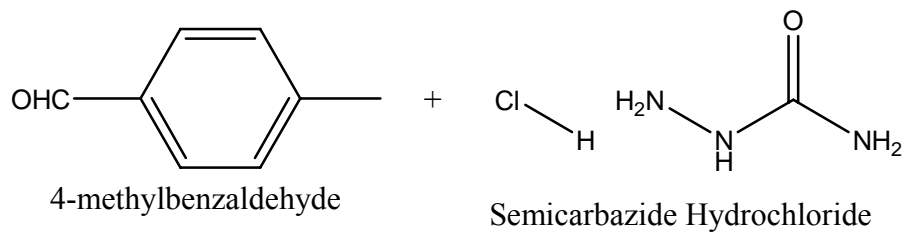


## STEP-II

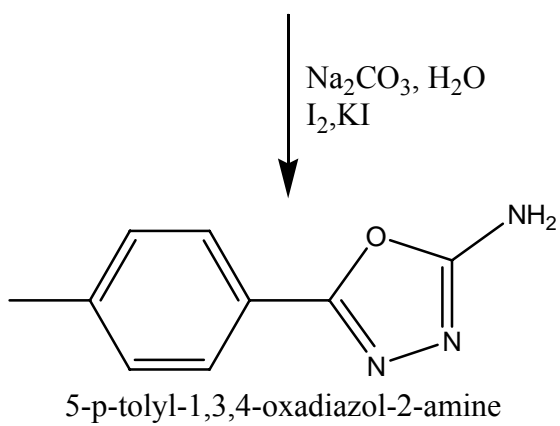


## SCHEME-III

## STEP-I

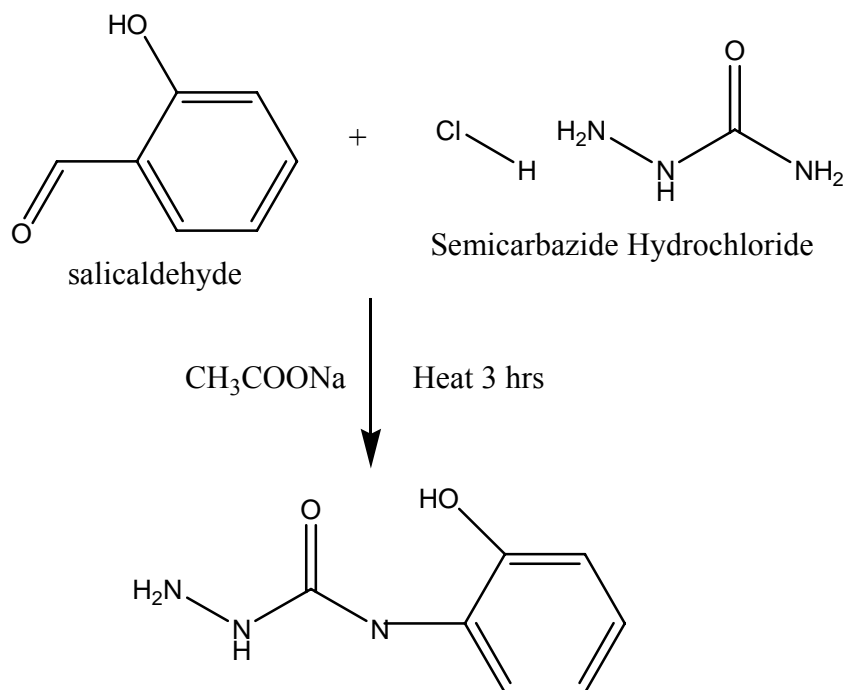


## STEP-II

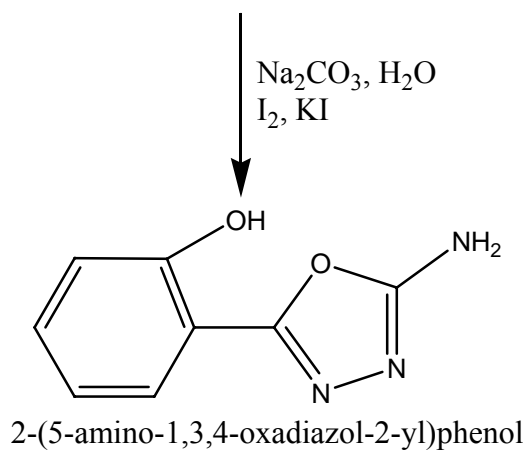


## SCHEME-IV

## STEP-I

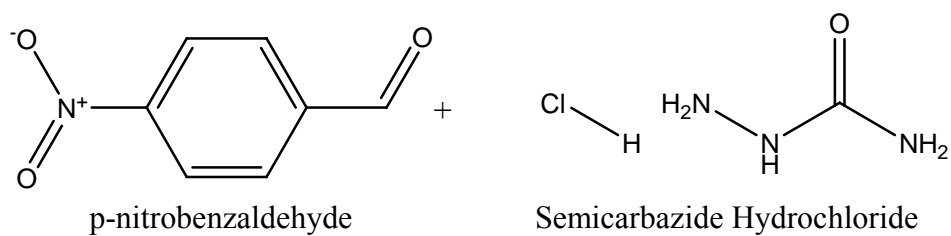


## STEP-II

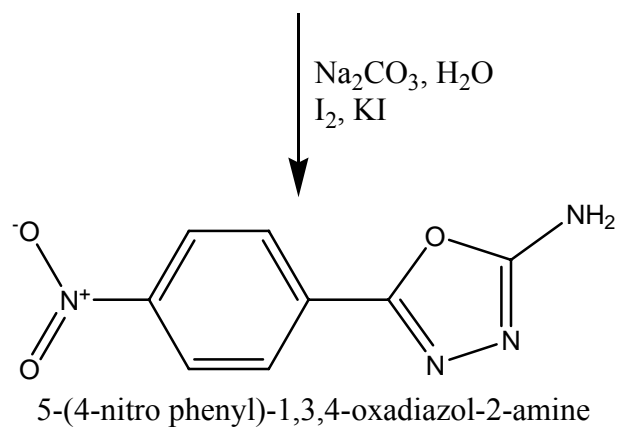


## SCHEME-V

## STEP-I

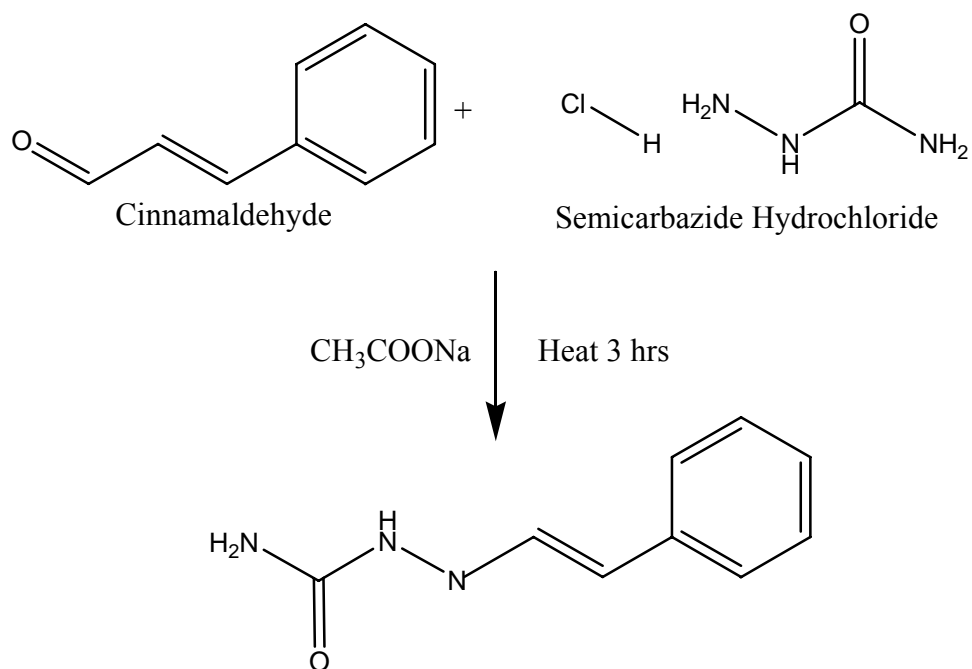


## STEP-II

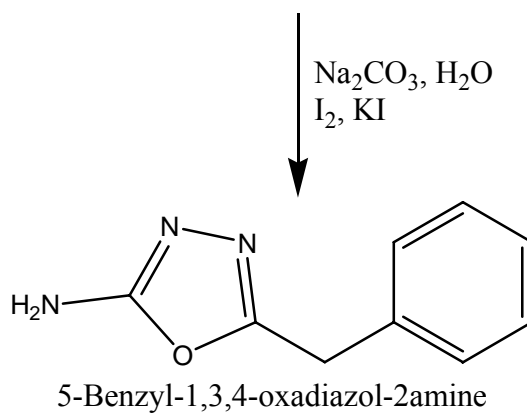


## SCHEME-VI

## STEP-I



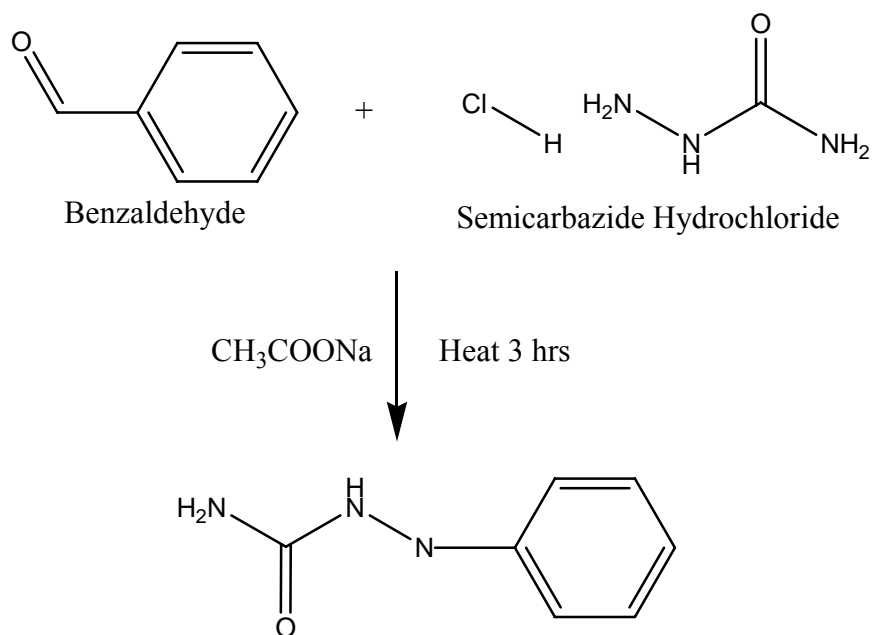
## STEP-II



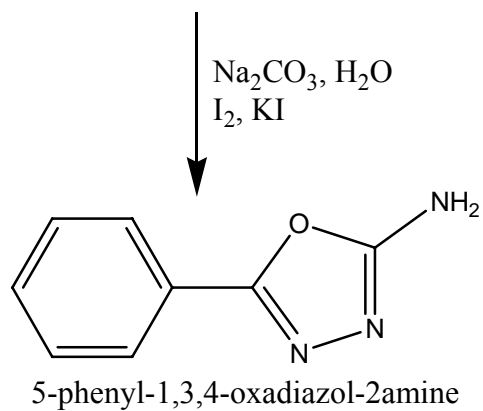


## SCHEME-VII

## STEP-I



## STEP-II



# *CHAPTER-IV*

## *PLAN OF WORK*

## 4. PLAN OF WORK

The proposed work was planned as follows:

### 1. Synthesis

- Synthesis of 1,3,4 oxadiazole derivatives

### 2. Study of physiochemical properties of the synthesized compounds

- Melting point, TLC and solubility profile of the synthesized compounds.

### 3. Characterization

- Characterization of the synthesized compounds by spectral studies (IR, HNMR and mass spectroscopy)

### 4. Screening of *Invitro* Anti inflammatory and Anthelmintic Activities

#### (a) Screening of *In-vitro* Anti inflammatory Activity

By HRBC Membrane Stabilization method.

#### (b) Screening of *In-vitro* Anthelmintic Activity

Anthelmintic activity of all synthesized compounds against *perionyx excavatus* and *perionyx sansibaricus* (earth worm) were evaluated.

*CHAPTER-V*

*MATERIALS  
&  
METHODS*

## 5. MATERIALS AND METHODS

### 5.1 EXPERIMENTAL

#### 5.1.1 SYNTHETIC METHOD

##### 5.1.1.1 CHEMICALS AND INSTRUMENTS

**Table II - Chemical List**

S.NO	CHEMICALS USED	NAME OF THE MANUFACTURERS
1.	Aldehyde Derivatives	
	a) Vanillin	Chemlabs
	b) Veratraldehyde	Hi-Media chemicals
	c) p-nitro benzaldehyde	Chemlabs
	d) Salisaldehyde	Chemlabs
	e) Cinnamaldehyde	Chemlabs
	f) Benzaldehyde	Chemlabs
	g) 4-methyl benzaldehyde	Chemlabs
2.	Semicarbazide Hydrochloride	Hi-Media chemicals
3.	Sodium Acetate	Chemlabs
4.	Iodine	Hi-Media chemicals
5.	Potassium Iodide	Hi-Media chemicals
6.	Ethanol	Hayman Limited, England
7.	Methanol	Hayman Limited, England
8.	Chloroform	Chemlabs
9.	Tetrahydrofuran	Hayman Limited, England
10.	Dimethyl Sulfoxide	Hi-Media chemicals

### Instruments

1. IR Spectroscopy – Shimadzu FT 8300
2. NMR Spectroscopy – JEOL GSX 400
3. Mass Spectroscopy – JEOL GCmate Spectrometer
4. UV Spectrophotometer – Shimadzu 1200

### 5.1.1.2 SYNTHESIS OF COMPOUNDS

#### (a) Preparation of 1,3,4 Oxadiazole Derivatives

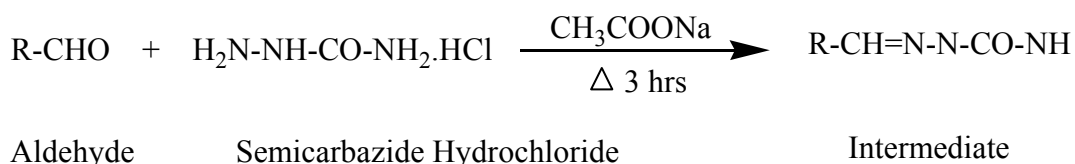
##### Step-I:

##### Chemicals Used

Aldehyde	-	0.03mole
Semicarbazide	-	0.05mole
Ethanol	-	20ml

##### Procedure

A mixture of aldehyde (0.03mole) and semicarbazide HCl (0.05mole) in ethanol (20ml) was refluxed for 3 hours at 100<sup>0</sup>C. Solvent was distilled off and the solid mass thus obtained was used for further reaction.



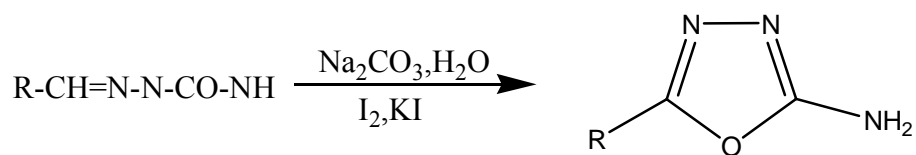
##### Step-II:

##### Chemicals Used

Solid Mass	-	0.01mole
Sodium Carbonate	-	0.01mole
Iodine	-	0.01mole
Potassium Iodide	-	0.01mole
Water	-	25ml

##### Procedure

The mixture of above solid mass (0.01mole) and sodium carbonate (0.01mole) was dissolved in water (25ml). Iodine (0.01mole) and potassium iodide (0.01mole) was refluxed for 2 hours at 100<sup>0</sup>C. The reaction mixture was then concentrated, allowed to cool, the solid product obtained was filtered, washed with water and re-crystallized using methanol.



Derivatives of 1,3,4-oxadiazole

The percentage yield, melting point, solubility and TLC profile were studied for all the seven synthesized compounds.

## **5.1.2 PHARMACOLOGICAL SCREENING**

### **5.1.2.1 ANTI INFLAMMATORY<sup>5</sup>**

These are the agents used to suppress the inflammation and pain sensation. The drugs used as anti-inflammatory and analgesic come under the class of non steroidal anti-inflammatory drugs and antipyretic and analgesic. All drugs grouped in this class have analgesic, antipyretic and anti-inflammatory action in different measures.

### **Inflammation**

Inflammation is defined as a defensive tissue reaction to infection, irritation or foreign substances. It is a part of the host defence mechanism but when it becomes great it is a hopeless condition. There are several tissue factors or mechanism that are known to be involved in the inflammatory reaction such as release of histamine, bradykinins and prostaglandins. Inflammation is the local response of living mammalian tissue to injury due to any agents. It is a body defence reaction in order to eliminate or limit the spread of injurious agent as well as to remove the consequent necrosed cells and tissues<sup>3</sup>.

### **Agents Causing Inflammation**

- Chemical agents like organic and inorganic poisons
- Physical agents like heat, cold, irradiation, mechanical trauma
- Infective agents like bacteria, viruses and their toxins
- Immunological agents like cell-mediated and antigen-antibody reactions.

### **Signs of Inflammation**

- Rubor (redness)
- Tumor (swelling)
- Color (heat)
- Dolor (pain)

### **Types of Inflammation**

Depending upon the defence capacity of the host and duration of response, inflammation can be classified as acute and chronic.

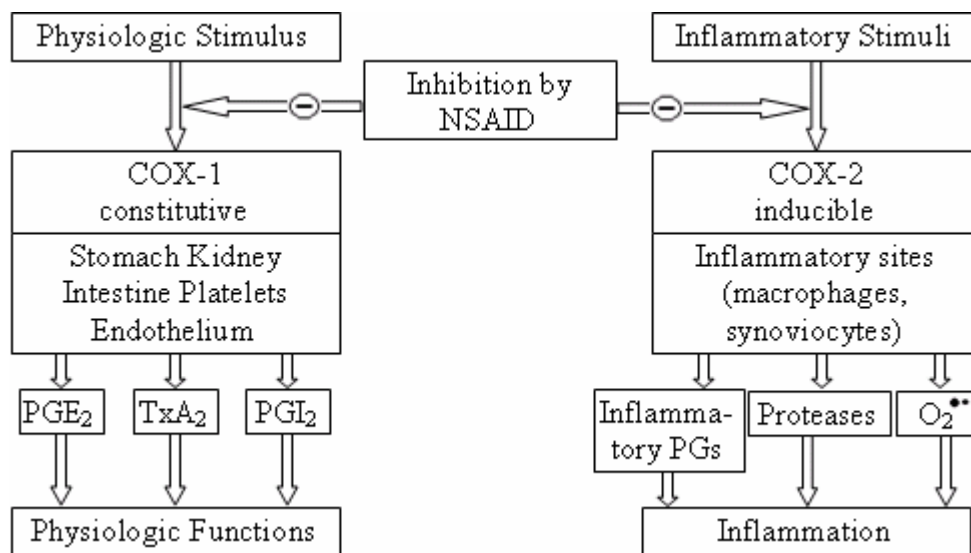


**Acute Inflammation** is short duration and represents the early body reaction and is usually followed by repair.

**Chronic Inflammation** is of longer duration and occurs either after the causative agent of acute inflammation persists for longer time or stimulus that it includes chronic inflammation from the beginning.

### Mechanism of Action<sup>6</sup>

The main action of NSAIDS is the inhibition of arachidonic acid-metabolizing activity of COX, as described originally by Vane in 1971. The cyclo oxygenase enzymes are bifunctional, having two distinct activities; the main action which gives PGG<sub>2</sub> and a peroxidase action, which converts PGG<sub>2</sub> to GH<sub>2</sub>. Both COX-1 and COX-2 inhibit only the cyclo oxygenation reaction. Both COX-1 and COX-2 are associated with the membrane and each consists of a long channel with a bend at the end, the channel being wider in COX-2. The opening of the channel is largely hydrophobic the arachidonic acid enters, is twisted round the bend and has two oxygen inserted in the 5-carbon ring characteristic of prostaglandins.



**Fig.1 - Mechanism of Action**

COX-1 inhibition, in general, is instantaneous and competitively reversible. COX-2 inhibition is time dependent, i.e., its effect increases with time. Other actions of inhibition of COX may contribute to the anti inflammatory effect of the same NSAIDS. Reaction oxygen radicals produced by neutrophils and macrophages are implicated in tissue damage in some condition and NSAIDS that particularly strong oxygen radical scavenging effects as well as COX-inhibiting activity (sulindac) may decrease tissue damage. Aspirin has been shown to inhibit expression of the transcription factor NF-KB, which has a key role in the transaction of the genes for inflammatory mediators.

So from this the literature reveals that 1,3,4-oxadiazole derivatives possess anti inflammatory activity. So an attempt was made to screen the synthesized compounds for anti inflammatory activity.

#### **5.1.2.2 *IN VITRO* ANTI INFLAMMATORY ACTIVITY<sup>10</sup>**

##### **Method**

The Human Red Blood Cells (HRBC) membrane stabilization has been used as a method to study the anti-inflammatory activity.

##### **Chemicals and Reagents Used**

- Diclofenac Sodium
- Hypo saline (0.36%)
- Isosaline (0.85%, pH 7.2)
- Phosphate Buffer (0.15M, pH 7.2)
- Alsever Solution

##### **Preparation of Alsever Solution**

Alsever solution was prepared by 2% dextrose, 0.8% sodium citrate, 0.05% citric acid and 0.42% sodium chloride dissolved in distilled water and sterilized.

##### **Procedure**

Blood was collected from healthy volunteers. The collected blood was mixed with equal volume of sterilized alsever solution.

The blood was centrifuged at 3000 rpm and packed cells were washed with isosaline and a 10% (v/v) suspension was made with isosaline.

Two solutions of each synthesized compounds were prepared (each containing 100 µg/ml) and to each solution added 1 ml phosphate buffer, 2ml of hyposaline and 0.5ml HRBC suspension diclofenac sodium was used as a reference drug. Instead of hypo saline, 2ml of distilled was used as control. The array mixtures were incubated at 37°C for 30 min and centrifuged. The hemoglobin content in supernatant solution was estimated using UV analysis at 560nm. The % hemoglobin was calculated by assuming the hemoglobin produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the formula

$$\% \text{ prevention of Hemoglobin} = 100 \times \frac{\text{O.D of Test-O.D of Product}}{\text{O.D of Control}}$$

The control represents 100% lysis. The result was compared with standard diclofenac (50 µg/ml).

### **5.1.2.2 *INVITRO* ANTHELMINTIC ACTIVITY<sup>11</sup>**

Anthelmintics or antihelminthics are drugs that expel parasitic worms (helminths) from the body, by either stunning or killing them. They may also be called vermifuges (stunning) or vermicides (killing).

#### **Anthelmintic Resistance**

The ability of worms to survive treatments that are generally effective at the recommended dose rate is considered a major threat to the future control of worm parasites of small ruminants and horses.

Treatment with an antihelminthic drug kills worms whose genotype renders them susceptible to the drug. Worms that are resistant survive and pass on their "resistance" genes. Resistant worms accumulate and finally treatment failure occurs.

### Types of Worm Species Found In India

- *Perionyx excavatus*
- *Perionyx sansibaricus*
- *Eisenia foetida*
- *Eisenia eugeniae*
- *Lampito mauritti*
- *Pontoscolex corethrurus*
- *Pheretima posthuma*
- *Octochaetona serrata*

### Materials Collected

Two different species of earth worms (*perionyx excavatus* and *perionyx sansibaricus*-obtained from Bell foundation, Thiruvannamiyur, Chennai-41) of nearly equal size were selected for present study.

*Perionyx excavatus*, *Perionyx sansibaricus* are commercially produced earthworms. They are also known as "blues" or "Indian blues". They belong to the *Perionyx* genus. Their origins may be the Himalayan Mountains. This species is particularly good for vermicomposting in tropical and subtropical regions.

Earth worms are washed with normal saline to remove all the faecal matter. The earth worms of 3-5cm in length and 0.1-0.2cm width were selected for the experimental protocol.

### Procedure

Anthelmintic activity<sup>12</sup> was carried out against *P.excavatus*, *P.sansibaricus* species at 2 mg/ml concentration. Suspension of samples was prepared by triturating synthesized compounds (100mg) with tween 80(0.5%) and distilled water and the resulting mixtures were stirred using a mechanical stirrer for 30 min. The suspensions were diluted to contain 0.2% w/v of the test samples. Suspension of reference drug and piperazine citrate was prepared with the same concentration in a similar way. Two sets of five earthworms of

almost similar sizes (2 inch in length) were placed in Petri plates of 4 inch diameter containing 10 ml suspension of test sample and reference drug. Another set of five earthworms was kept as control in 10 ml suspension of distilled water and Tween 80(0.5%). The paralyzing and death times were noted and their means were calculated. The death time was ascertained by placing the earthworms in warm water (50°C) which stimulated the movement, if the worm was alive. The results are presented in Table XI.

# *CHAPTER-VI*

## *RESULTS & DISCUSSION*

## 6. RESULTS AND DISCUSSION

At present in the scheme, the 1, 3, 4 oxadiazole derivatives were synthesized by using different aldehyde with yields between 70-83%. The molecular structure and IUPAC name for all seven synthesized compounds are tabulated in Table-III.

The solubility and TLC profile were studied for all the seven synthesized compounds and the results are tabulated in Table IV and V.

The melting points of all synthesized compounds were found in open capillary tubes and readings were uncorrected. The elemental analysis was determined and the results are tabulated in table VI. The found values of the elements by elemental analysis were closer to calculated values.

The IR spectra of the compounds were done in a shimadzu FT 8300 infrared spectrophotometer ( $V_{\max} \text{ cm}^{-1}$ ) by using KBr discs. The results of IR spectra are given in the table VII and it shows that the functional groups such as phenolic hydroxyl, carbonyl, amino, nitro phenyl and methoxy groups may be present in the synthesized compounds.

The mass spectra of all the synthesized compounds were done on a JEOL GC mate spectrometer. The results presented in the table VIII shows that the molecular mass of the synthesized compounds was nearer to the molecular mass of the expected compounds.

The  $^1\text{H}$ NMR spectra of the synthesized 1, 3, 4 oxadiazole derivative were recorded on JEOL GSX400 spectrometer using TMS as internal standard (chemical shifts in  $\delta$ , PPM) and DMSO as the solvent. The results of  $^1\text{H}$ NMR spectra given in table IX shows that the numbers of hydrogen atoms present in all the synthesized compounds were exact when compared to the number of hydrogen atoms in the expected compounds.

The synthesized compounds were screened for their *invitro* anti-inflammatory activity by membrane stabilization method. The results are given in table XI. All the synthesized compounds showed good anti-inflammatory activity. Out of all the synthesized compounds 1A, 1B, 1E showed significant anti-inflammatory activity when compared with

that of standard where as the compound 1C, 1D, 1F, 1G showed less when compared to that of standard diclofenac.

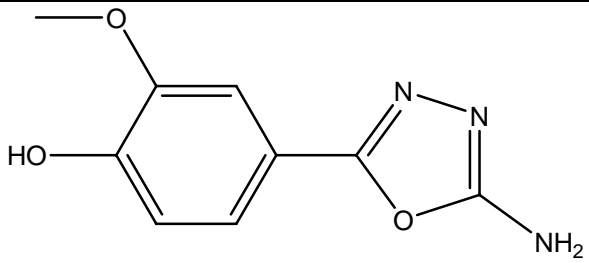
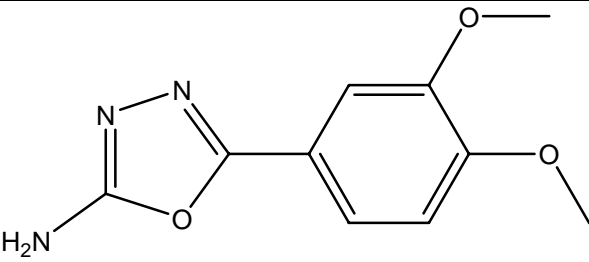
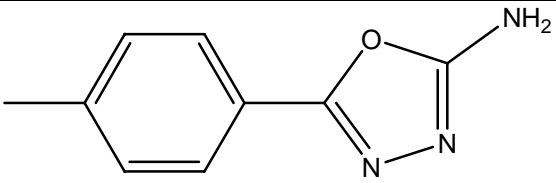
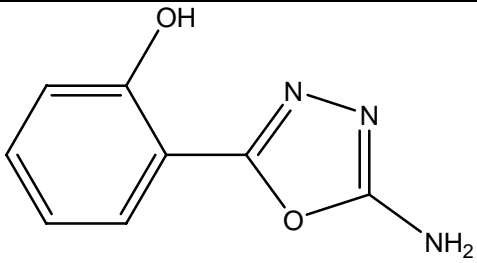
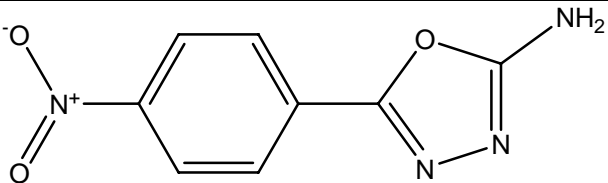
The literature shows that the compounds having methoxy,nitro,hydroxyl groups possess significant anti inflammatory activity<sup>22</sup>when compared to other groups .The synthesized compound 1A,1B,1E possess OCH<sub>3</sub>,OH,nitro group in its structure. So the anti inflammatory activity may be due to the presence of the respective functional groups as evidence in literature.

The anthelmintic activity was carried out using two species of earth worms. The synthesized compounds showed good anthelmintic property the activity may be due to presence of methyl and nitro group in the compound which has been proved in the earlier report<sup>12</sup>.

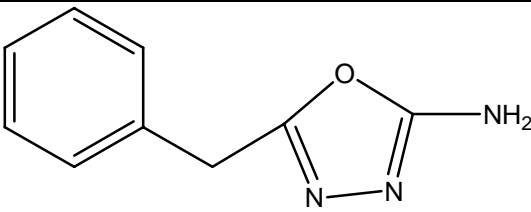
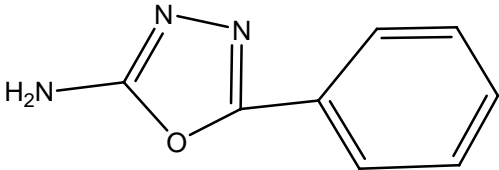
The paralyzing and death times were noted and their means are calculated which were tabulated in Table XI.



Table III - List of Compounds Synthesized 1A-1G

COMPOUNDS	STRUCTURE	IUPAC NAME
1A		4-(5-amino-1,3,4-oxadiazol-2-yl)-2-methoxyphenol
1B		5-(3,4-dimethoxyphenyl)-1,3,4-oxadiazol-2-amine
1C		5-p-tolyl-1,3,4-oxadiazol-2-amine
1D		2-(5-amino-1,3,4-oxadiazol-2-yl)phenol
1E		5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine



<b>1F</b>		5-Benzyl-1,3,4-oxadiazol-2-amine
<b>1G</b>		5-phenyl-1,3,4-oxadiazol-2-amine

**TABLE IV - Solubility data of the synthesized compound 1A-1G**

<b>COMPOUNDS</b>	<b>WATER</b>	<b>ACETONE</b>	<b>CHLOROFORM</b>	<b>DMSO</b>	<b>ETHANOL</b>	<b>METHONAL</b>	<b>ETHYL ACETATE</b>	<b>GLACIAL ACETIC ACID</b>	<b>DMF</b>
<b>1A</b>	-	-	++	++	+	++	-	-	+
<b>1B</b>	-	-	+	++	+	+	-	-	+
<b>1C</b>	-	-	++	++	+	+	-	-	+
<b>1D</b>	-	-	++	++	+	+	-	-	+
<b>1E</b>	-	-	++	++	+	+	-	-	+
<b>1F</b>	-	-	+	++	+	+	-	-	+
<b>1G</b>	-	-	+	++	+	+	-	-	+

++ = Freely Soluble  
+ = Slightly Soluble  
- = Insoluble

Table V - TLC Profile of the Synthesized Compounds

S.NO	COMPOUND	SOLVENT RATIO		R <sub>f</sub> VALUE
		CHLOROFORM	METHANOL	
1	1A	50	50	0.70
2	1B	70	30	0.73
3	1C	60	40	0.71
4	1D	70	30	0.75
5	1E	50	50	0.68
6	1F	50	50	0.76
7	1G	70	30	0.78

Adsorbent Used : Pre coated Silica gel G

Mobile Phase : Chloroform and Methanol

Detection Techniques : UV Chamber

**Table VI - Elemental Analysis Data of Compounds 1A-1F**

S.No	Compound	Mol. Formula	m.p <sup>0</sup> C	Molecular Weight	Yield in Percentage	Elemental Analysis of Compounds (%) Found			
						C	H	N	O
1	<b>1A</b>	C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O <sub>3</sub>	119	207.19	76	52.17	4.38	20.28	23.17
2	<b>1B</b>	C <sub>10</sub> H <sub>11</sub> N <sub>3</sub> O <sub>3</sub>	132	221.21	83	54.29	5.01	19.00	21.70
3	<b>1C</b>	C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O	140	175.19	70	61.70	5.18	23.99	9.13
4	<b>1D</b>	C <sub>8</sub> H <sub>7</sub> N <sub>3</sub> O <sub>2</sub>	147	177.16	75	54.24	3.98	23.72	18.06
5	<b>1E</b>	C <sub>8</sub> H <sub>6</sub> N <sub>4</sub> O <sub>3</sub>	136	206.16	80	46.61	2.93	27.18	23.28
6	<b>1F</b>	C <sub>9</sub> H <sub>9</sub> N <sub>3</sub> O	145	175.19	82	61.70	5.18	23.99	9.13
7	<b>1G</b>	C <sub>8</sub> H <sub>7</sub> N <sub>3</sub> O	150	161.16	76	59.62	4.38	26.07	9.93

**Table VII - IR Spectral data of the synthesized compounds 1A-1G**

<b>Compounds</b>	<b>C-H(Ar) (cm<sup>-1</sup>)</b>	<b>C=O (cm<sup>-1</sup>)</b>	<b>C-H (s) (cm<sup>-1</sup>)</b>	<b>C=C (cm<sup>-1</sup>)</b>	<b>C-N (cm<sup>-1</sup>)</b>	<b>C=N (cm<sup>-1</sup>)</b>	<b>C-H (b) (cm<sup>-1</sup>)</b>	<b>C-O- CH<sub>3</sub> (cm<sup>-1</sup>)</b>	<b>Phenolic OH (s) (cm<sup>-1</sup>)</b>	<b>Phenolic OH (b) (cm<sup>-1</sup>)</b>	<b>C-NO<sub>2</sub> (cm<sup>-1</sup>)</b>	<b>C-NH<sub>2</sub> (cm<sup>-1</sup>)</b>	<b>C-Cl (cm<sup>-1</sup>)</b>
<b>1A</b>	2771	-	-	1536	1434	1680	1140	-	3864	1230	-	3915	-
<b>1B</b>	3250	-	-	1502	1486	1692	1114	-	-	-	-	3930	-
<b>1C</b>	2794	-	-	1370	1299	1658	1154	-	-	-	-	3750	-
<b>1D</b>	2696	-	-	1400	1269	1621	1158	-	3843	1215	-	3343	-
<b>1E</b>	2869	-	-	1452	1405	1673	1211	-	-	-	1464	3841	-
<b>1F</b>	3185	-	-	1299	1269	1692	1164	-	-	-	-	3217	-
<b>1G</b>	3644	-	-	1277	1230	1683	1121	-	-	-	-	3372	-

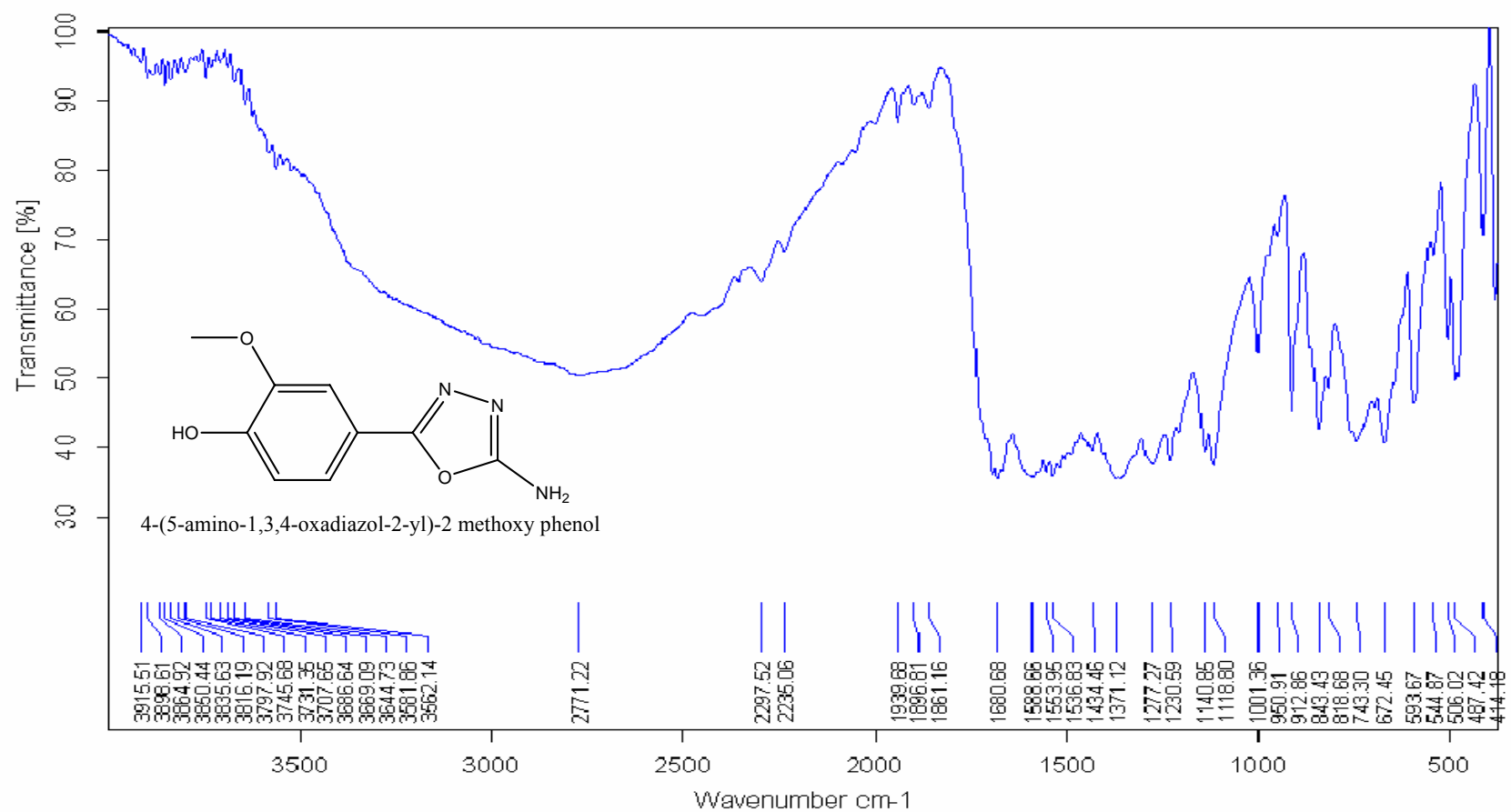
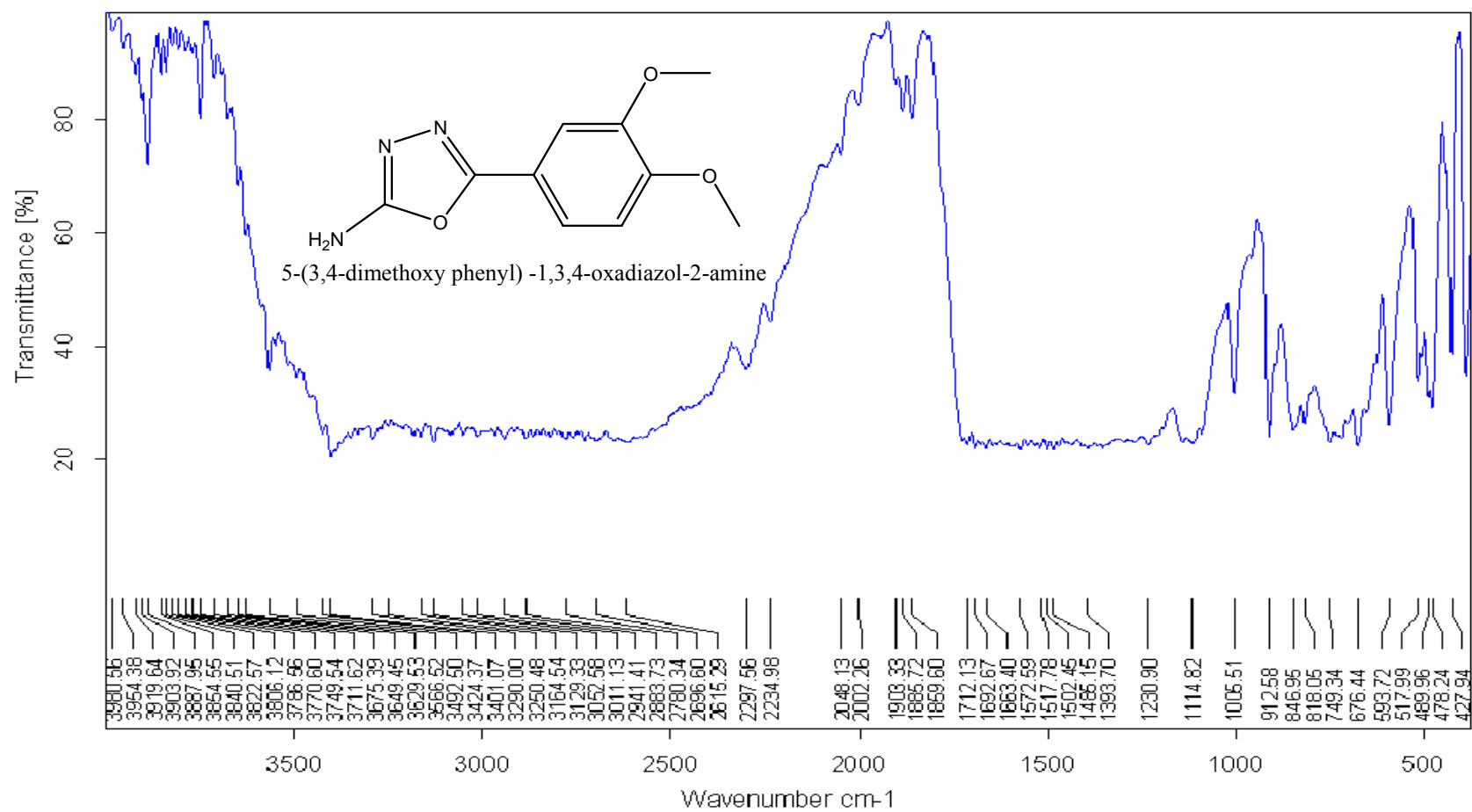


Fig.2



**Fig. 3**

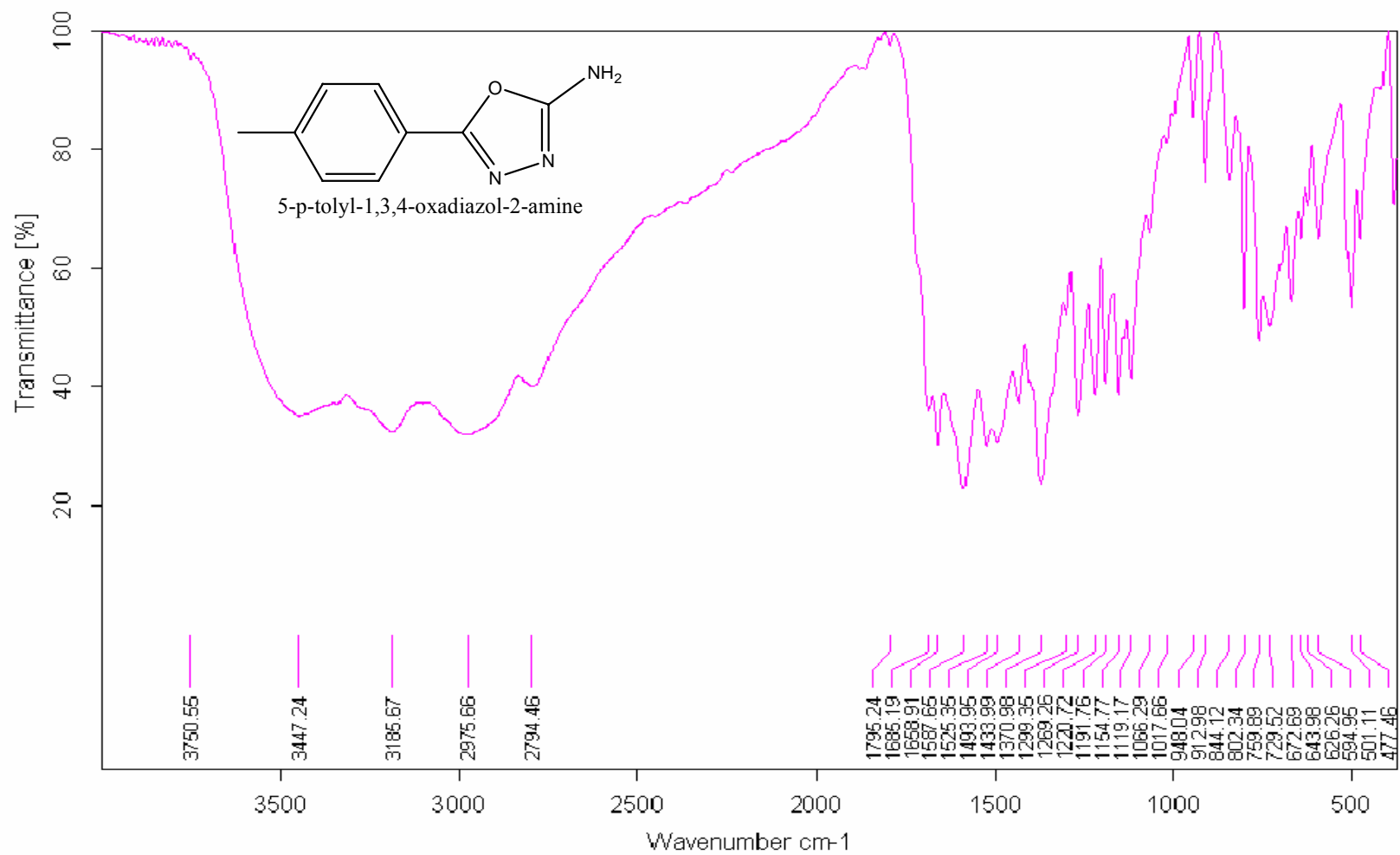


Fig. 4

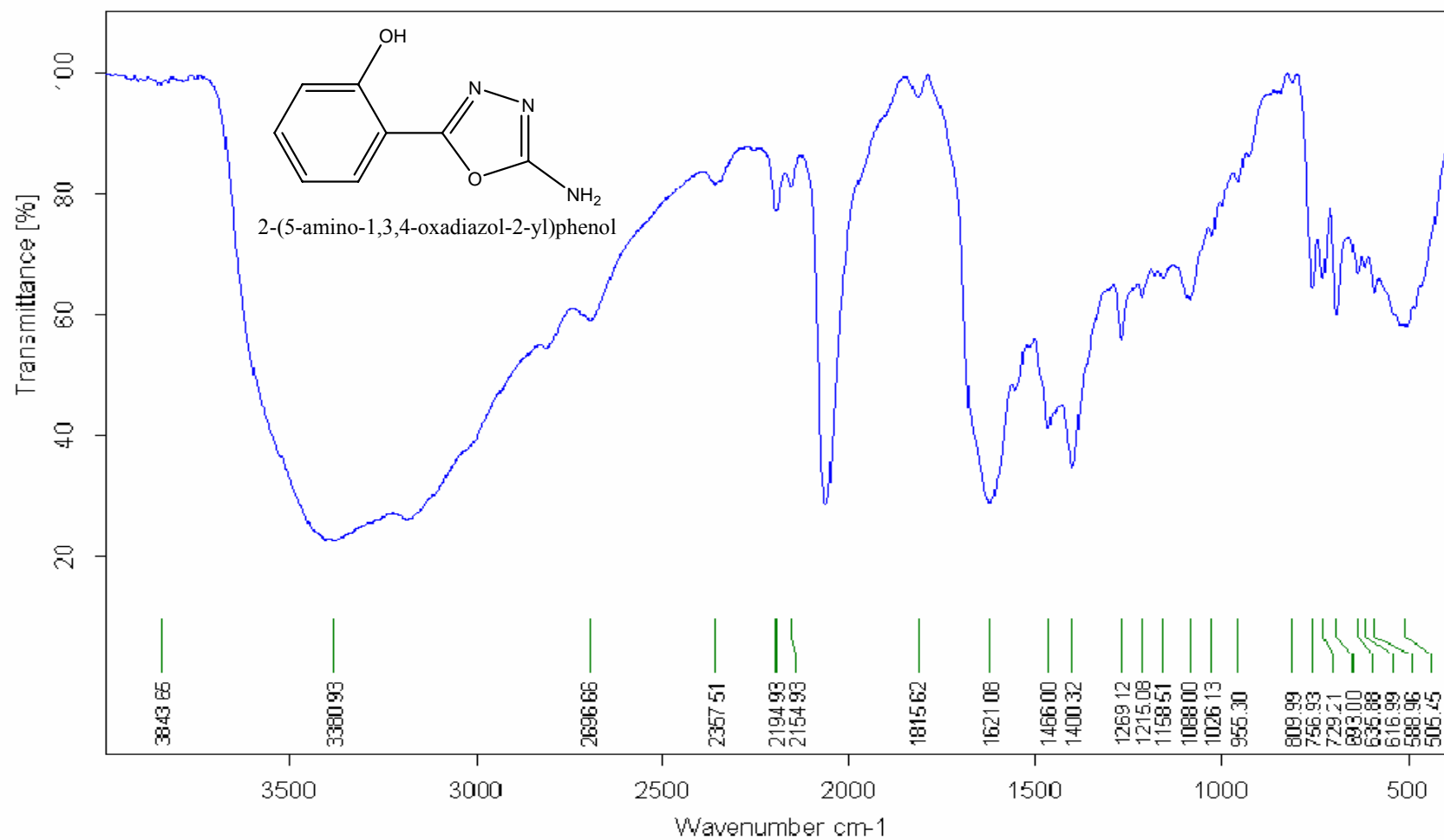


Fig. 5

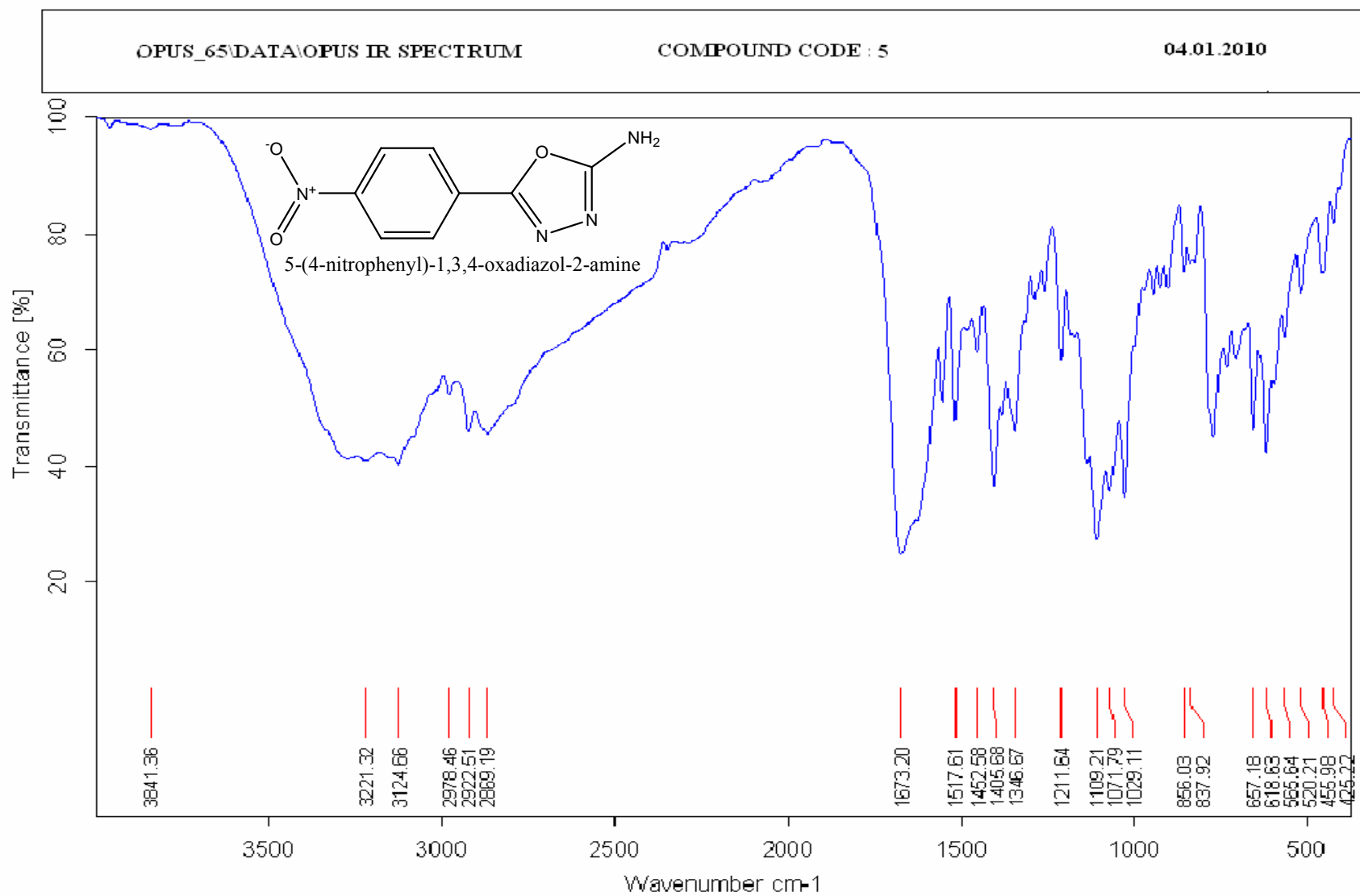


Fig. 6

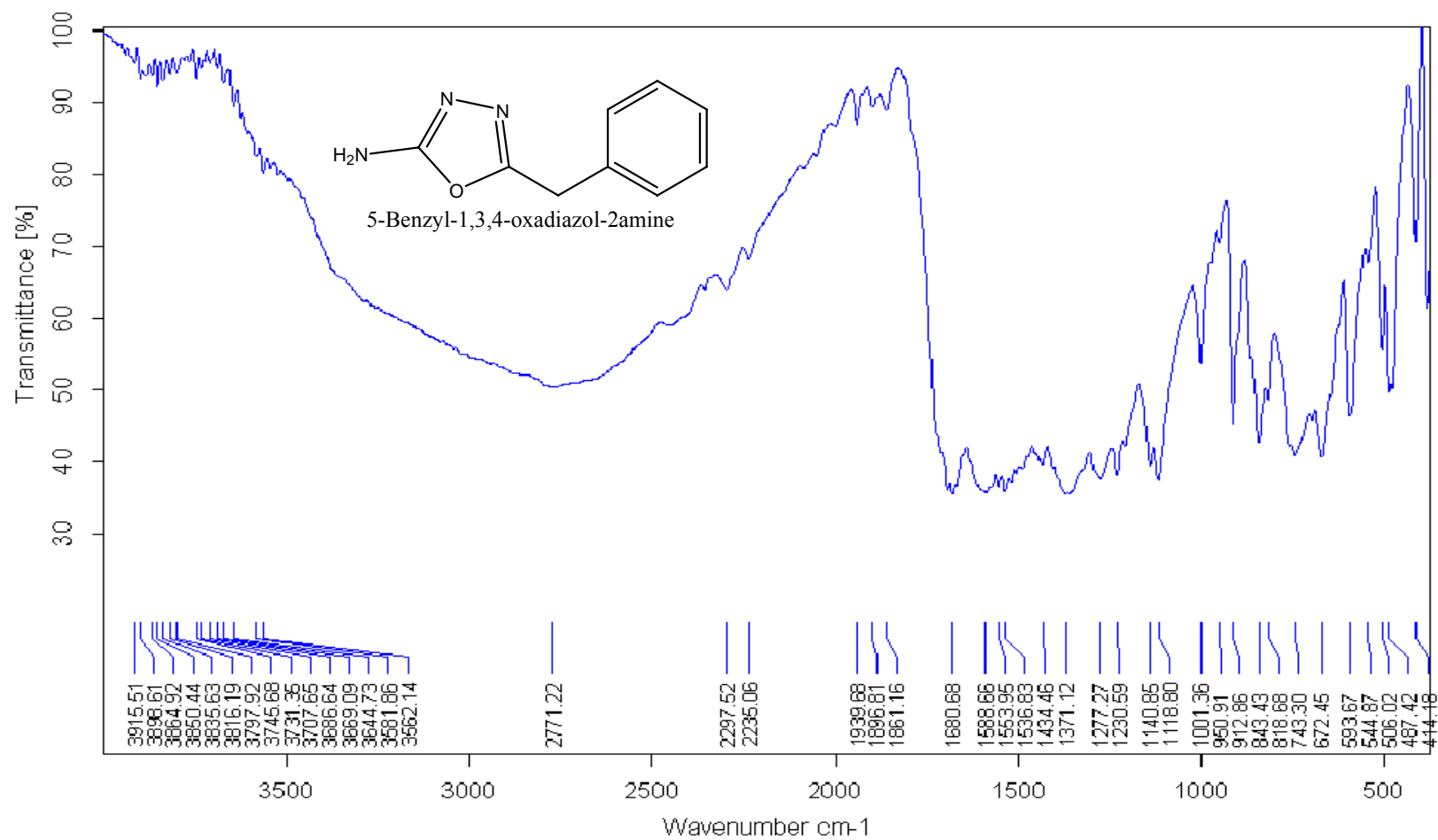


Fig.7

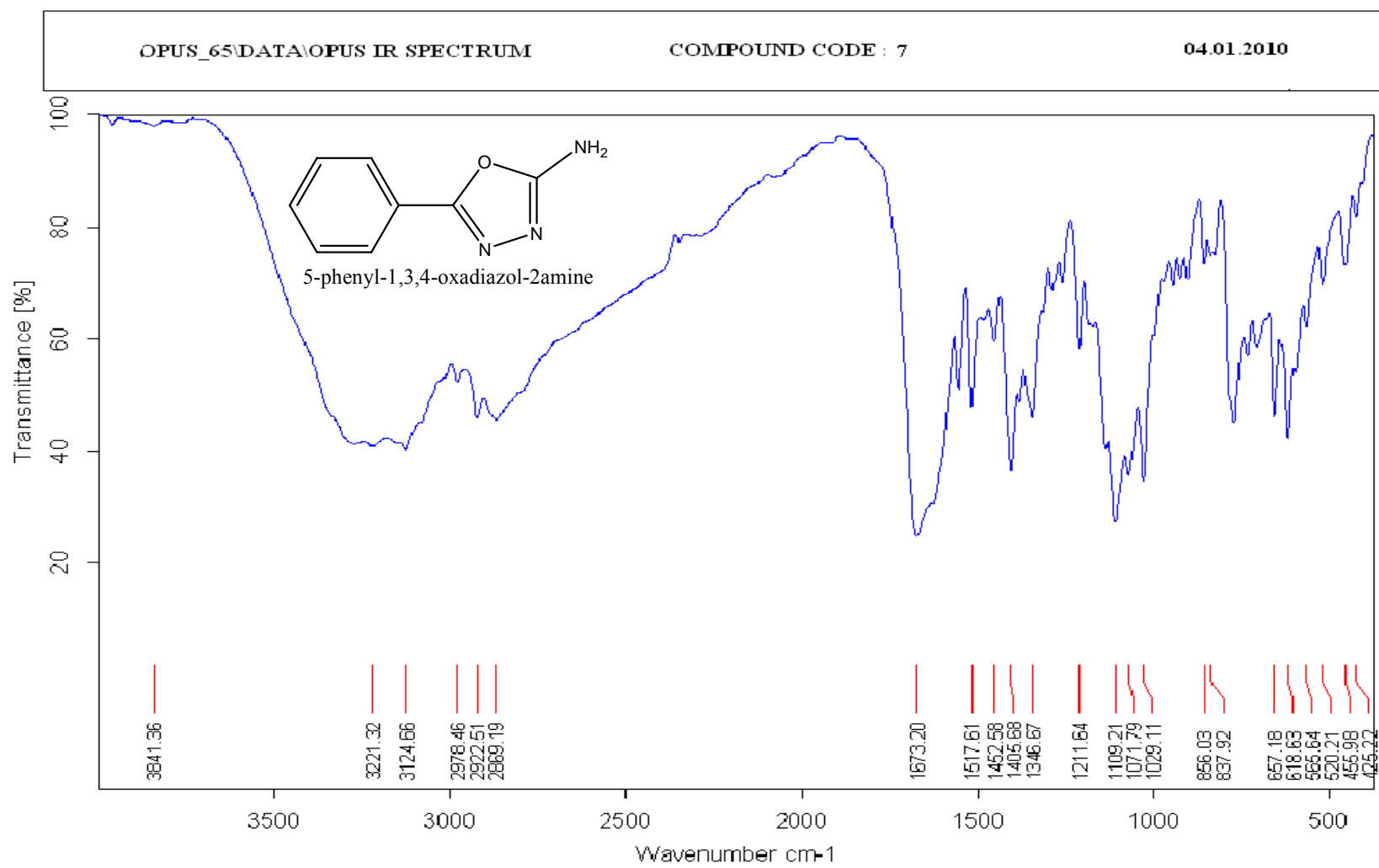


Fig. 8

**Table VIII – MASS Spectral data of the Synthesized Compounds 1A-1G**

S.No	Compounds	Molecular Weight	M/Z (% Relative Abundance)
1	<b>1A</b>	207.19	209.53 <b>M</b> <sup>+</sup> (35%), 202.57 <b>B</b> (155%), 166.45(55%), 149.44(100%), 134.40(55%), 124.43(30%), 117.42(15%), 106.38(30%), 93.39(20%), 69.36(17%), 58.38(35%)
2	<b>1B</b>	221.21	221.12 <b>M</b> <sup>+</sup> (45%), 149.4 <b>B</b> (100%), 134.40(50%), 106.38(36%), 93.39(29%), 58.34(38%)
3	<b>1C</b>	175.19	175.19 <b>M</b> <sup>+</sup> (50%), 119.42 <b>B</b> (100%), 61.32(100%), 91.38(75%), 107.43(45%), 77.37(55%), 69.38 (19%), 73.36(18%), 83.40(17%), 102.38(20%), 122.42(81%)
4	<b>1D</b>	177.16	177.49 <b>M</b> <sup>+</sup> ( 42%), 133.43 <b>B</b> (100%), 117.42(99%), 61.33(98%), 91.38(55%), 106.41(50%), 77.35(38%), 65.34(22%), 69.37(10%)
5	<b>1E</b>	206.16	206.16 <b>M</b> <sup>+</sup> ( 25%), 166.45 <b>B</b> (50%), 149.44(100%), 134.40(55%), 124.43(30%), 106.38(32%), 93.39(20%), 83.40(10%), 69.36(15%), 65.34(35%)
6	<b>1F</b>	175.19	177.49 <b>M</b> <sup>+</sup> ( 40%), 133.43 <b>B</b> (100%), 117.42(99%), 61.33(98%), 91.38(55%), 106.41(50%), 77.35(38%), 65.34(26%), 69.37(7%), 103.411(15%)
7	<b>1G</b>	161.16	162.47 <b>M</b> <sup>+</sup> ( 20%), 135.42 <b>B</b> (50%), 119.42(100%), 91.38(75%), 77.37(50%), 58.34(55%), 69.38(10%), 73.36(7%), 122.42(12%), 129.48(5%)

Indian Institute of Technology, Chennai  
12/08/2009

File: Bharathi 1A

Date Run: 12-08-2009 (Time Run: 16:24:11)

Sample:

Instrument: JEOL GCmate

Inlet: Direct Probe

Ionization mode: E1+

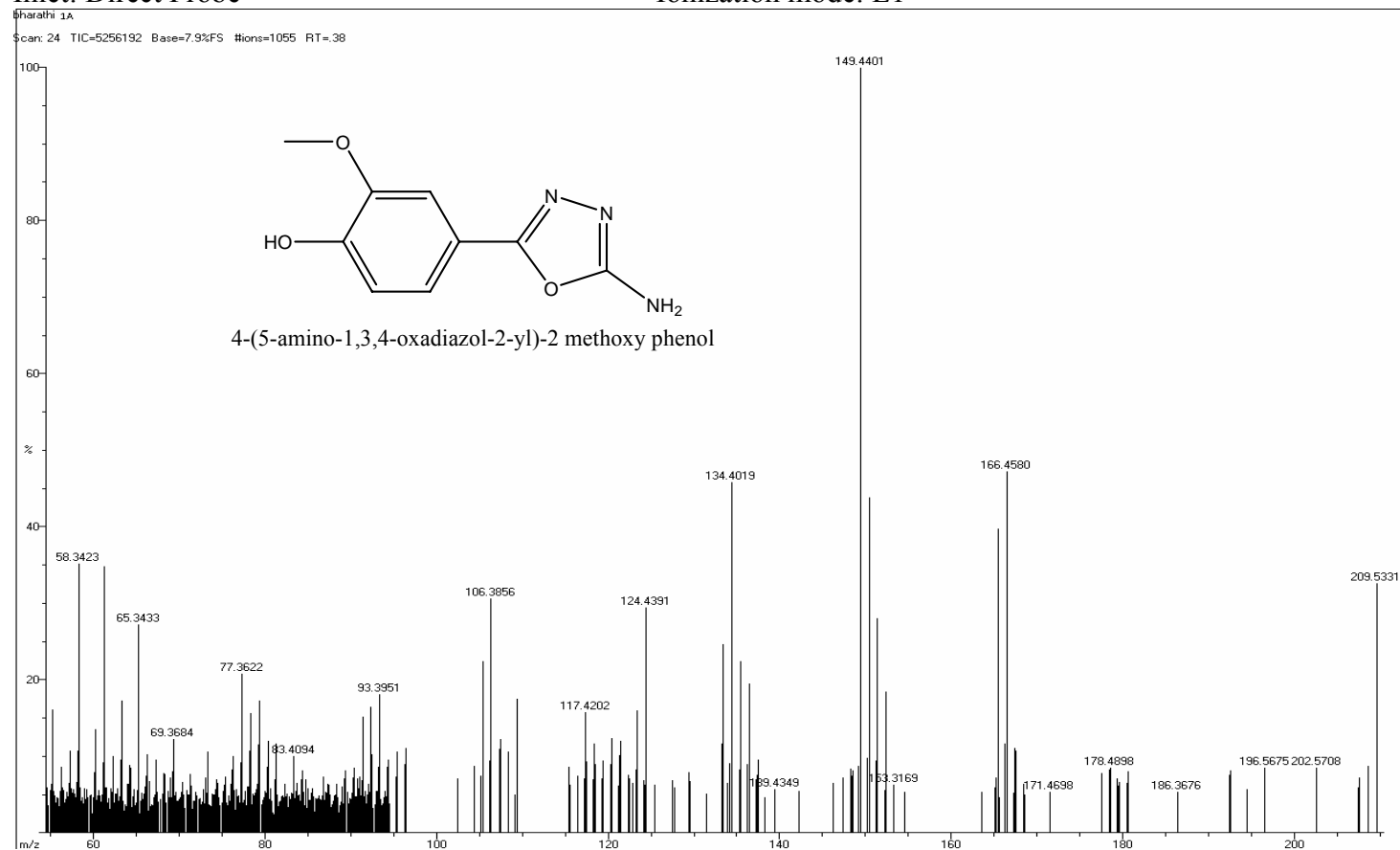


Fig. 9



Indian Institute of Technology, Chennai  
12/08/2009

File: Bharathi 1B

Date Run: 12-08-2009 (Time Run: 16:28:15)

Sample:

Instrument: JEOL GCmate

Inlet: Direct Probe

Ionization mode: E1+

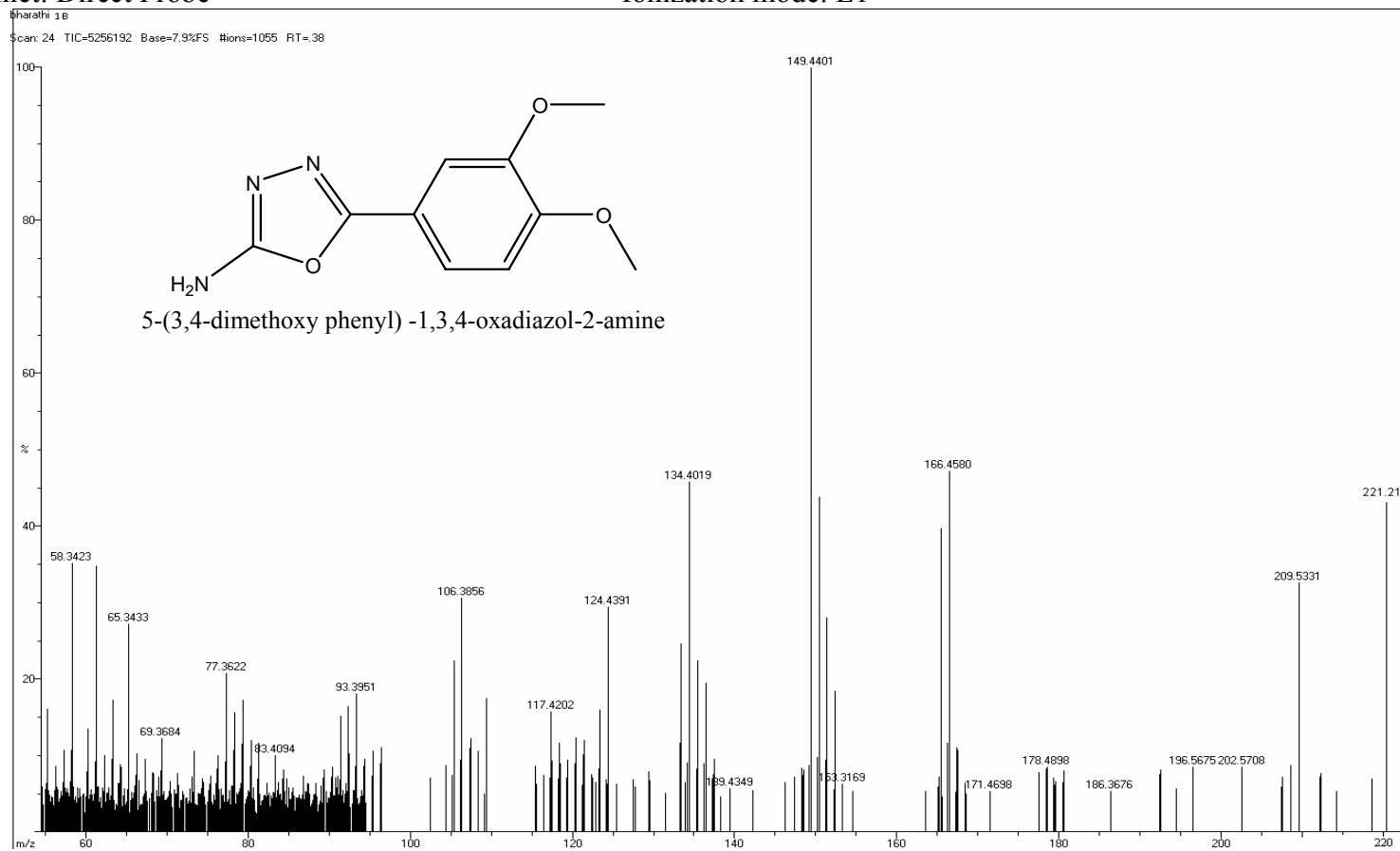


Fig. 10

Indian Institute of Technology, Chennai  
12/08/2009

File: Bharathi 1C

Date Run: 12-08-2009 (Time Run: 16:30:21)

Sample:

Instrument: JEOL GCmate

Inlet: Direct Probe

Ionization mode: E1+

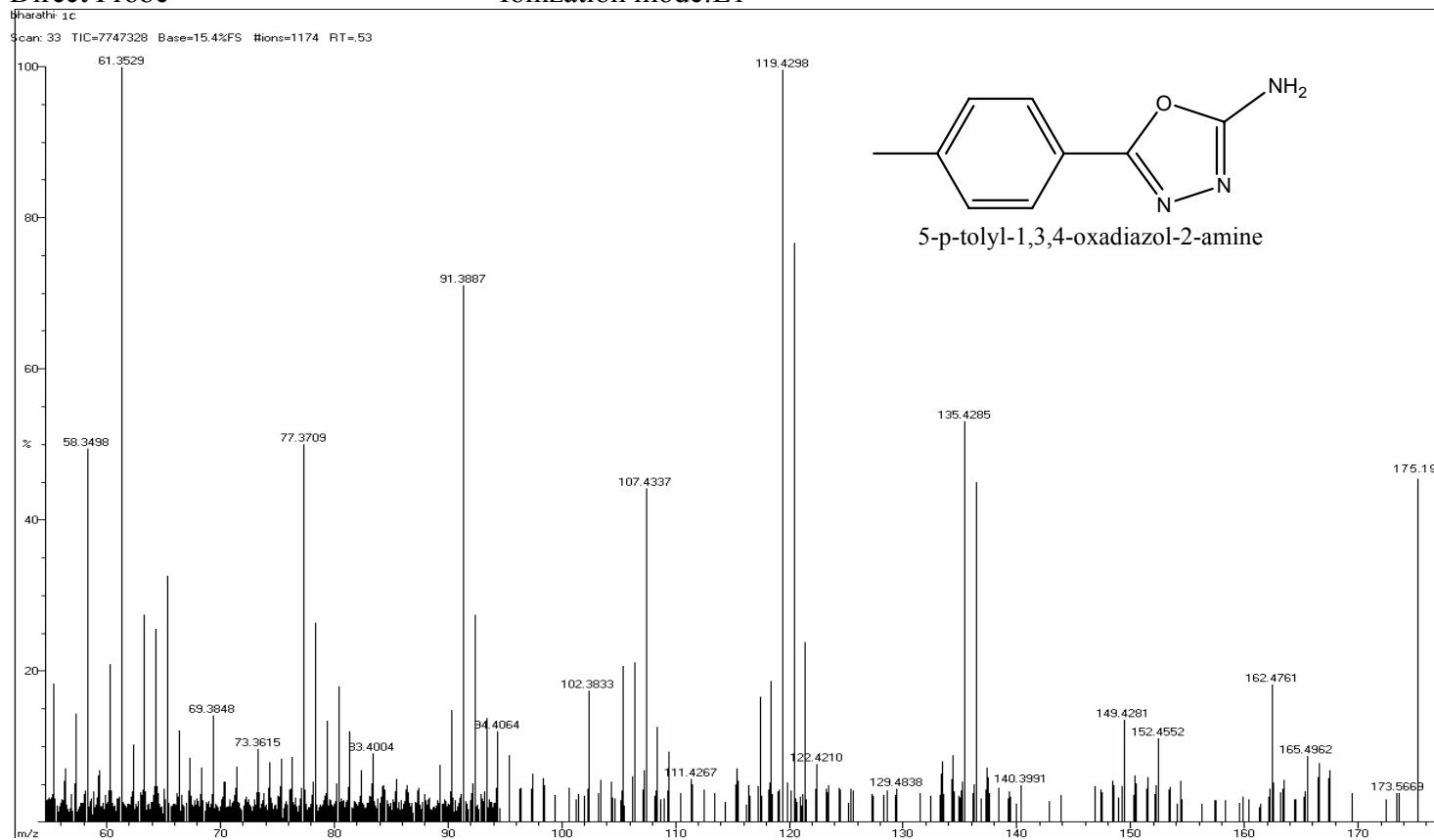


Fig.11

Indian Institute of Technology, Chennai  
12/08/2009

File: Bharathi 1D

Date Run: 12-08-2009 (Time Run: 16:32:20)

Sample:

Instrument: JEOL GCmate

Inlet: Direct Probe

Ionization mode: E1+

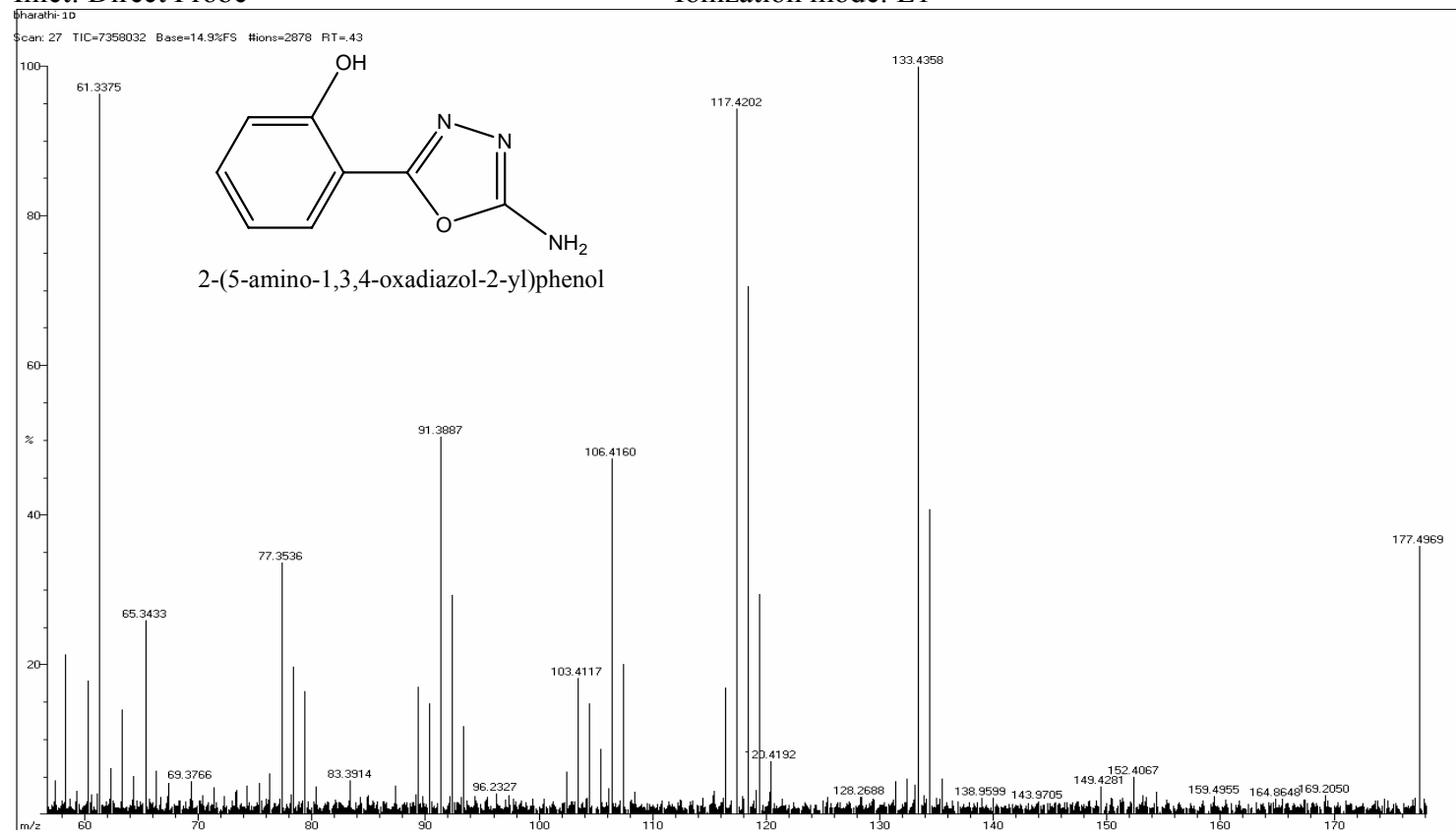


Fig. 12

Indian Institute of Technology, Chennai  
12/08/2009

File: Bharathi 1E

Date Run: 12-08-2009 (Time Run: 16:34:01)

Sample:

Instrument: JEOL GCmate

Inlet: Direct Probe

Ionization mode: E1+

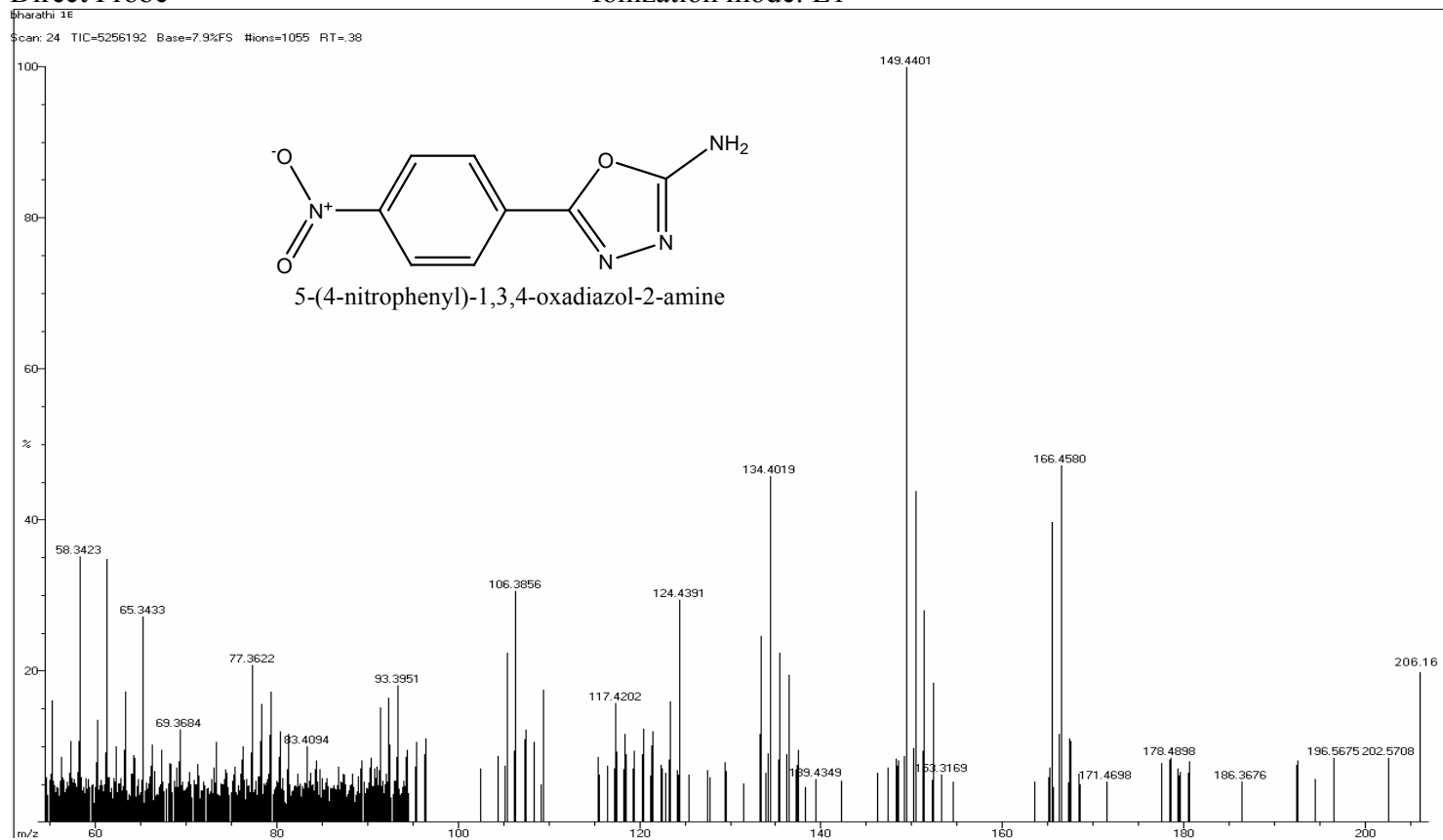


Fig. 13

Indian Institute of Technology, Chennai  
12/08/2009

File: Bharathi 1F

Date Run: 12-08-2009 (Time Run: 16:36:15)

Sample:

Instrument: JEOL GCmate

Inlet: Direct Probe

Ionization mode: E1+

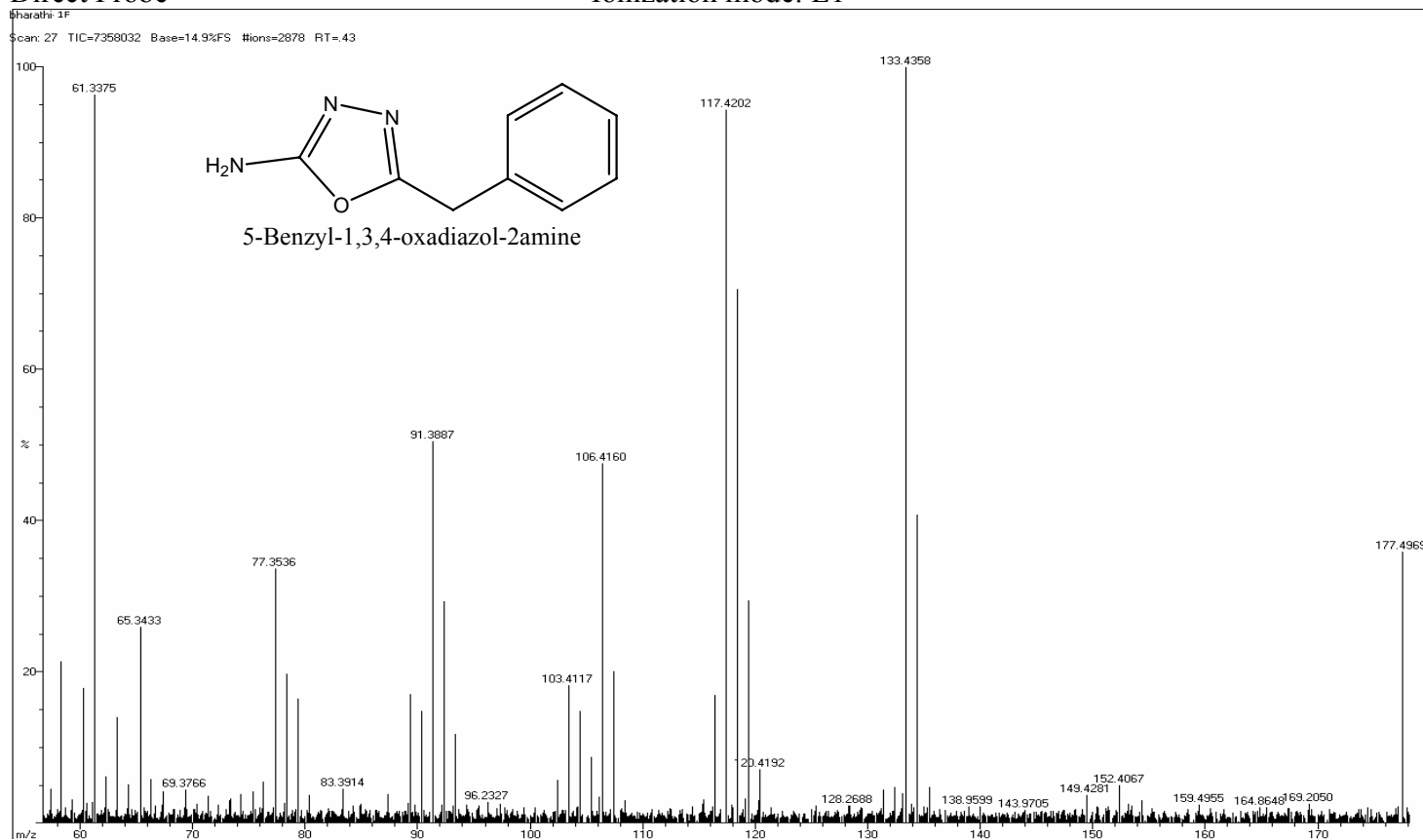


Fig. 14

Indian Institute of Technology, Chennai  
12/08/2009

File: Bharathi 1G

Date Run: 12-08-2009 (Time Run: 16:38:55)

Sample:

Instrument: JEOL GCmate

Inlet: Direct Probe

Ionization mode: E1+

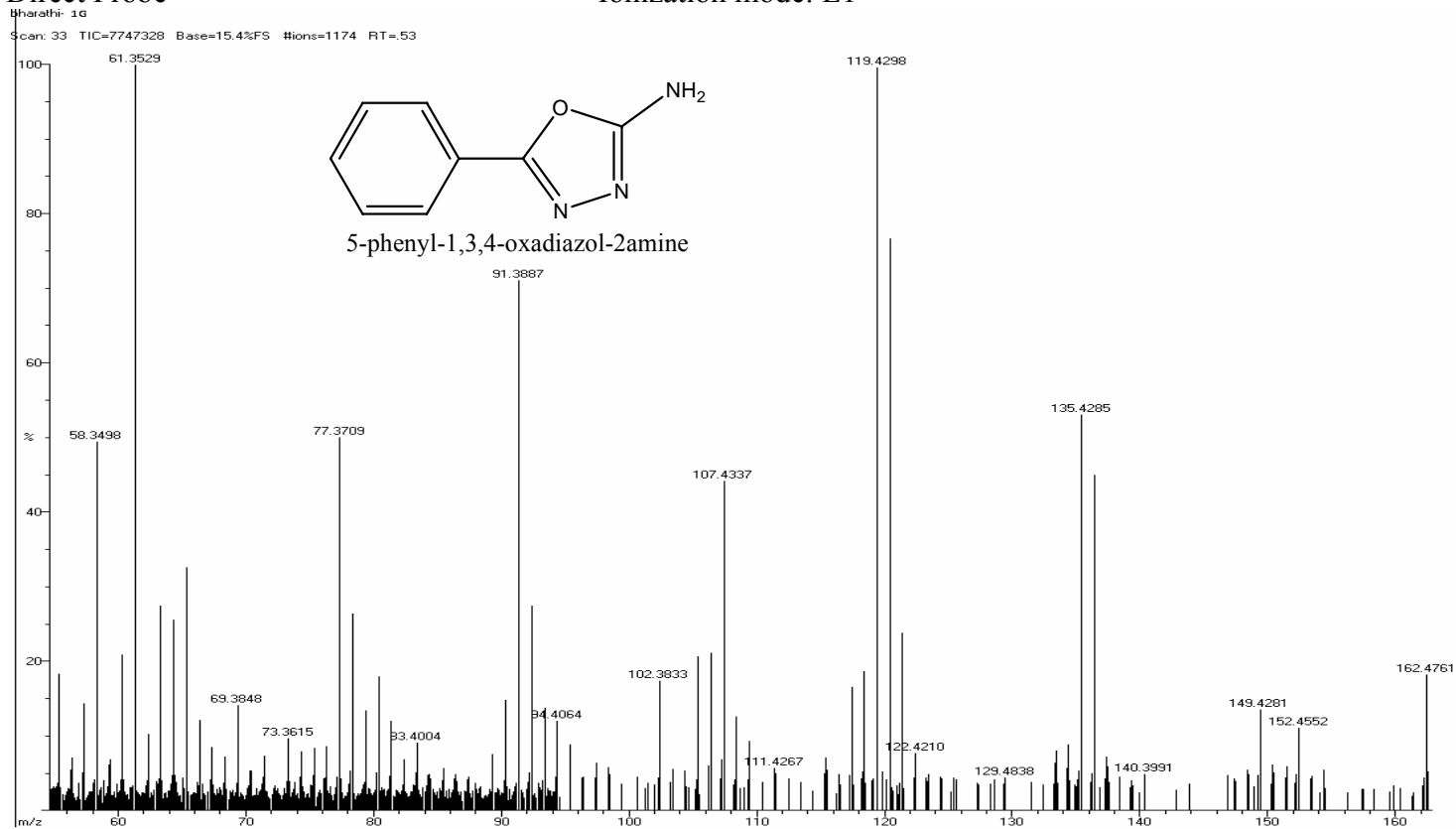
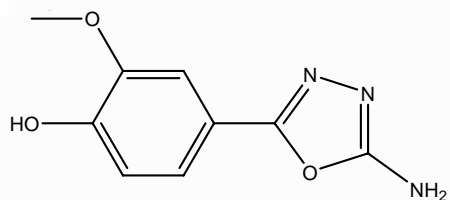


Fig. 15

**Table IX - <sup>1</sup>HNMR Spectral Data of the Synthesized Compounds 1A-1F**

[illegible]

1A.....Bharathi



4-(5-amino-1,3,4-oxadiazol-2-yl)-2 methoxy phenol

**BRUKER AVIII 500 MHz NMR Facility**  
SAIF, IIT Madras Chennai 600 036

Current Data Parameters  
NAME Dec31-2009  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date 20091231  
Time 16.14  
INSTRUM spect  
PROBHD 5 mm PABBO BB-  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.315264 Hz  
AQ 1.5860212 sec  
RG 80.6  
DW 48.400 usec  
DE 6.50 usec  
TE 298.1 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
NUC1 1H  
P1 10.65 usec  
PL1 0.00 dB  
PL1W 23.53637505 W  
SFO1 500.1330885 MHz

F2 - Processing parameters  
SI 32768  
SF 500.1300000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

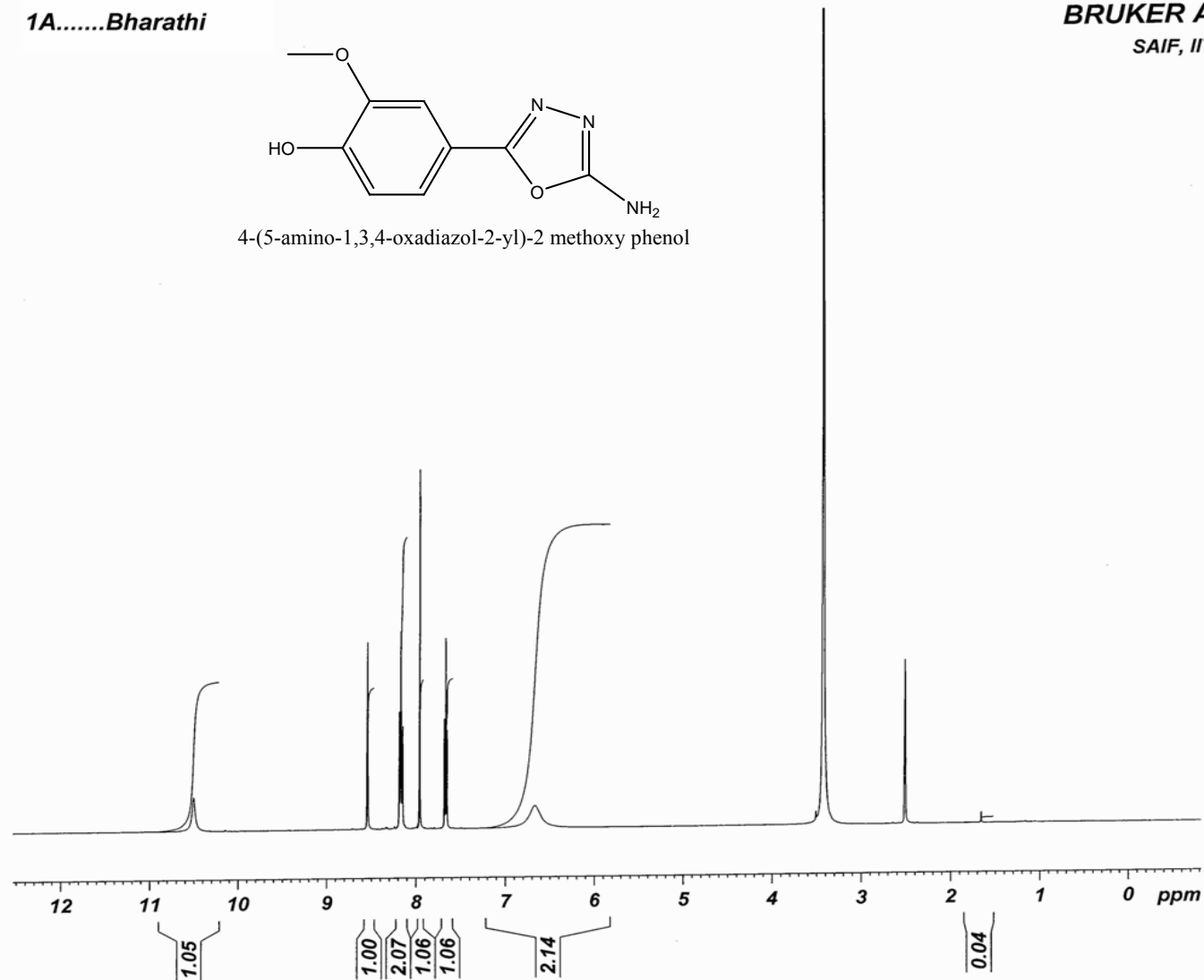
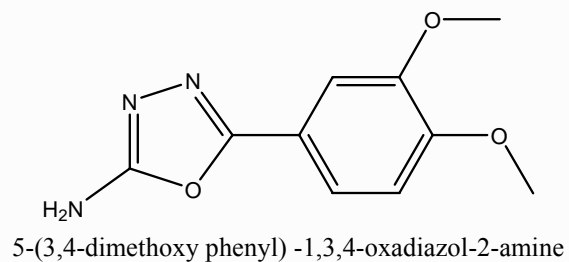


Fig. 16



1B.....Bharathi



**BRUKER AVIII 500 MHz NMR Facility**  
**SAIF, IIT Madras Chennai 600 036**

Current Data Parameters  
NAME Dec31-2009  
EXPNO 3  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20091231  
Time 16.18  
INSTRUM spect  
PROBHD 5 mm PABBO BB-  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.315264 Hz  
AQ 1.5860212 sec  
RG 64  
DW 48.400 usec  
DE 6.50 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
NUC1 1H  
P1 10.65 usec  
PL1 0.00 dB  
PL1W 23.53637505 W  
SFO1 500.1330885 MHz

F2 - Processing parameters  
SI 32768  
SF 500.130000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

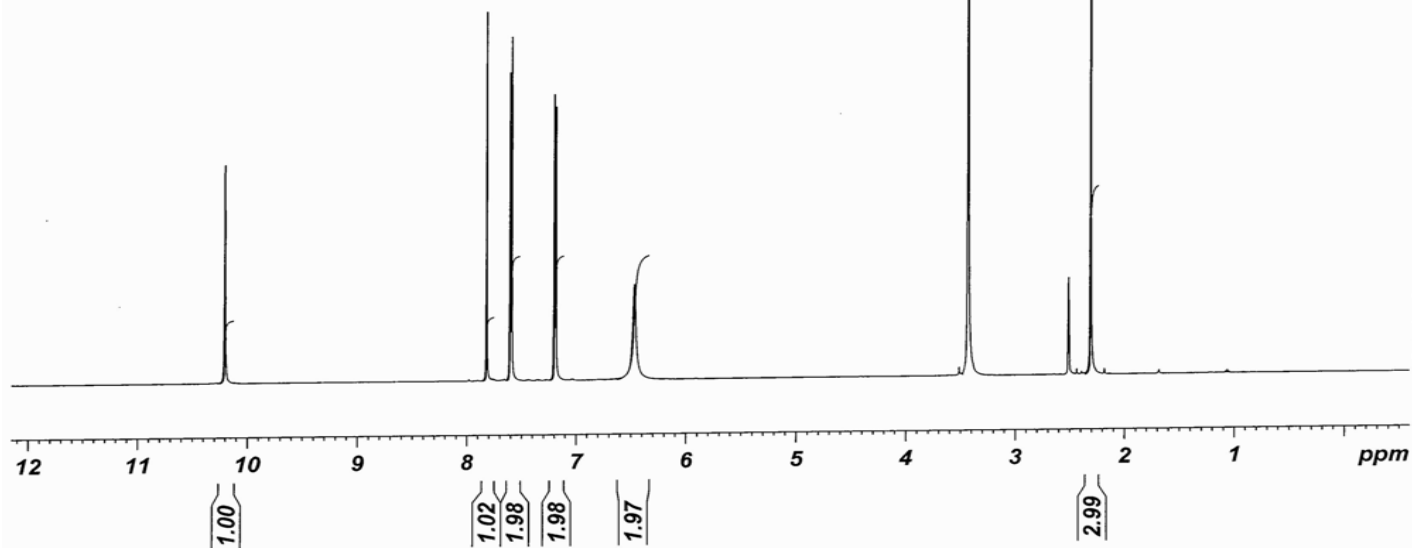
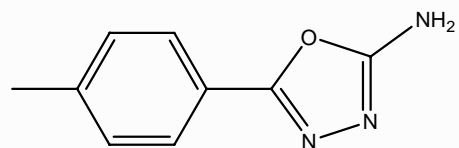


Fig. 17

1C.....Bharathi



5-p-tolyl-1,3,4-oxadiazol-2-amine

SAIF, IIT Madras

Current Data Parameters  
NAME Dec31-2009  
EXPNO 5  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20091231  
Time 16.26  
INSTRUM spect  
PROBHD 5 mm PABBO BB-  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.315264 Hz  
AQ 1.5860212 sec  
RG 71.8  
DW 48.400 usec  
DE 6.50 usec  
TE 297.9 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
NUC1 1H  
P1 10.65 usec  
PL1 0.00 dB  
PL1W 23.53637505 W  
SFO1 500.1330885 MHz

F2 - Processing parameters  
SI 32768  
SF 500.1300000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

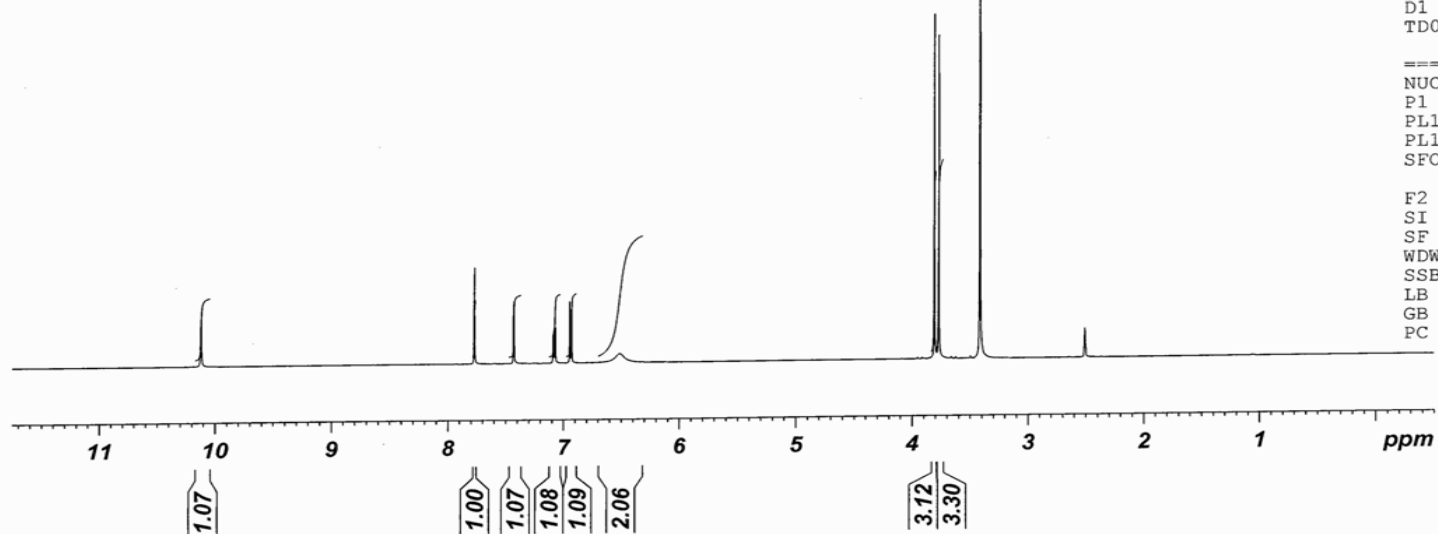
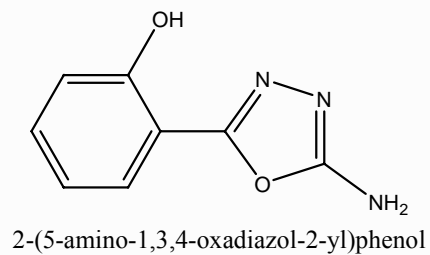


Fig. 18

1D.....Bharathi



SAIF, IIT Madras

Current Data Parameters  
NAME Dec31-2009  
EXPNO 4  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20091231  
Time\_ 16.22  
INSTRUM spect  
PROBHD 5 mm PABBO BB-  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.315264 Hz  
AQ 1.5860212 sec  
RG 101  
DW 48.400 usec  
DE 6.50 usec  
TE 298.0 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
NUC1 1H  
P1 10.65 usec  
PL1 0.00 dB  
PL1W 23.53637505 W  
SFO1 500.1330885 MHz

F2 - Processing parameters  
SI 32768  
SF 500.1300000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

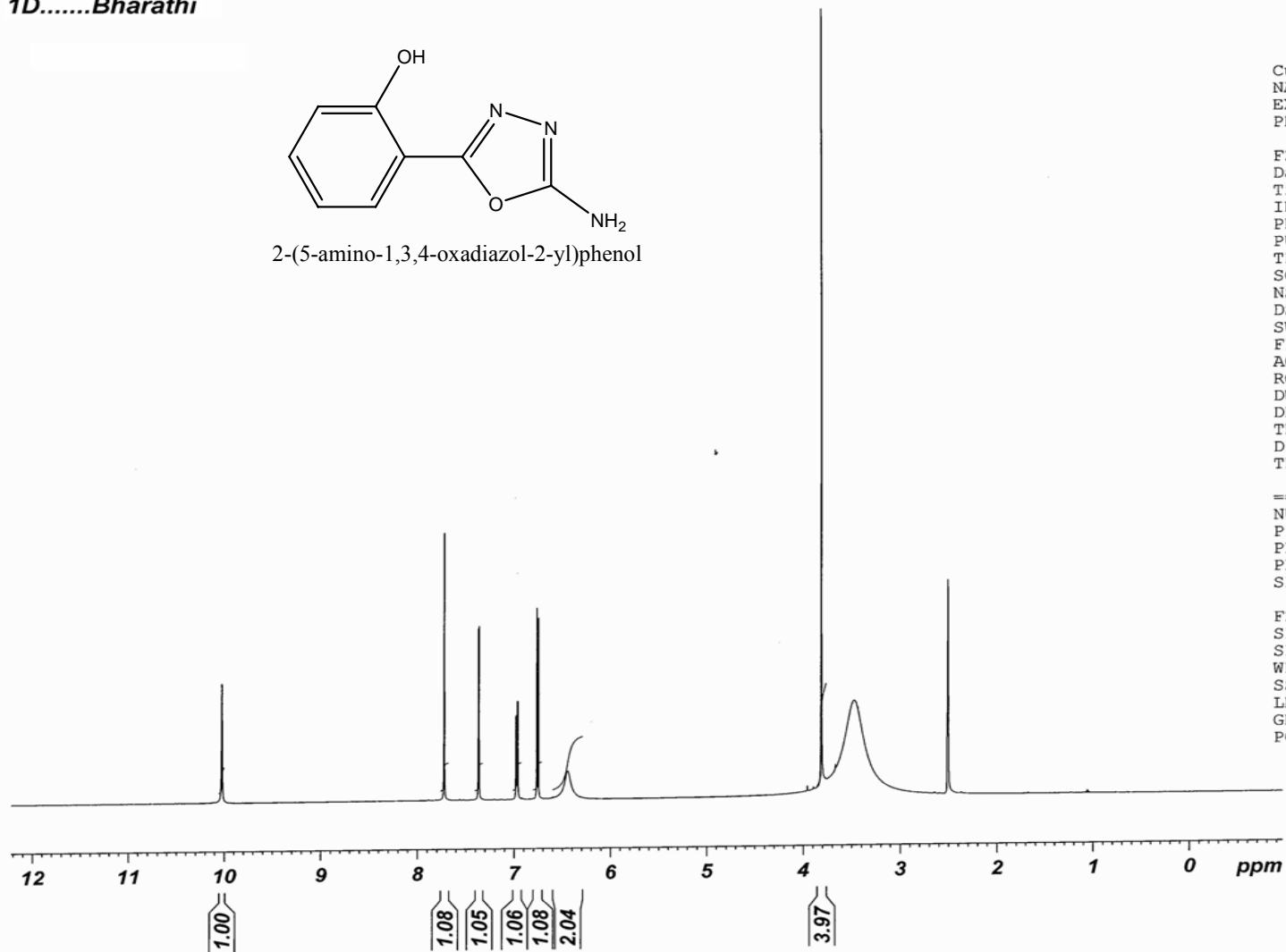
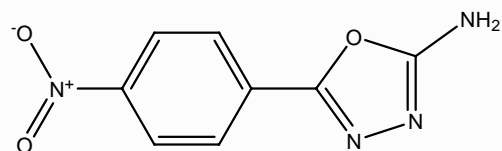


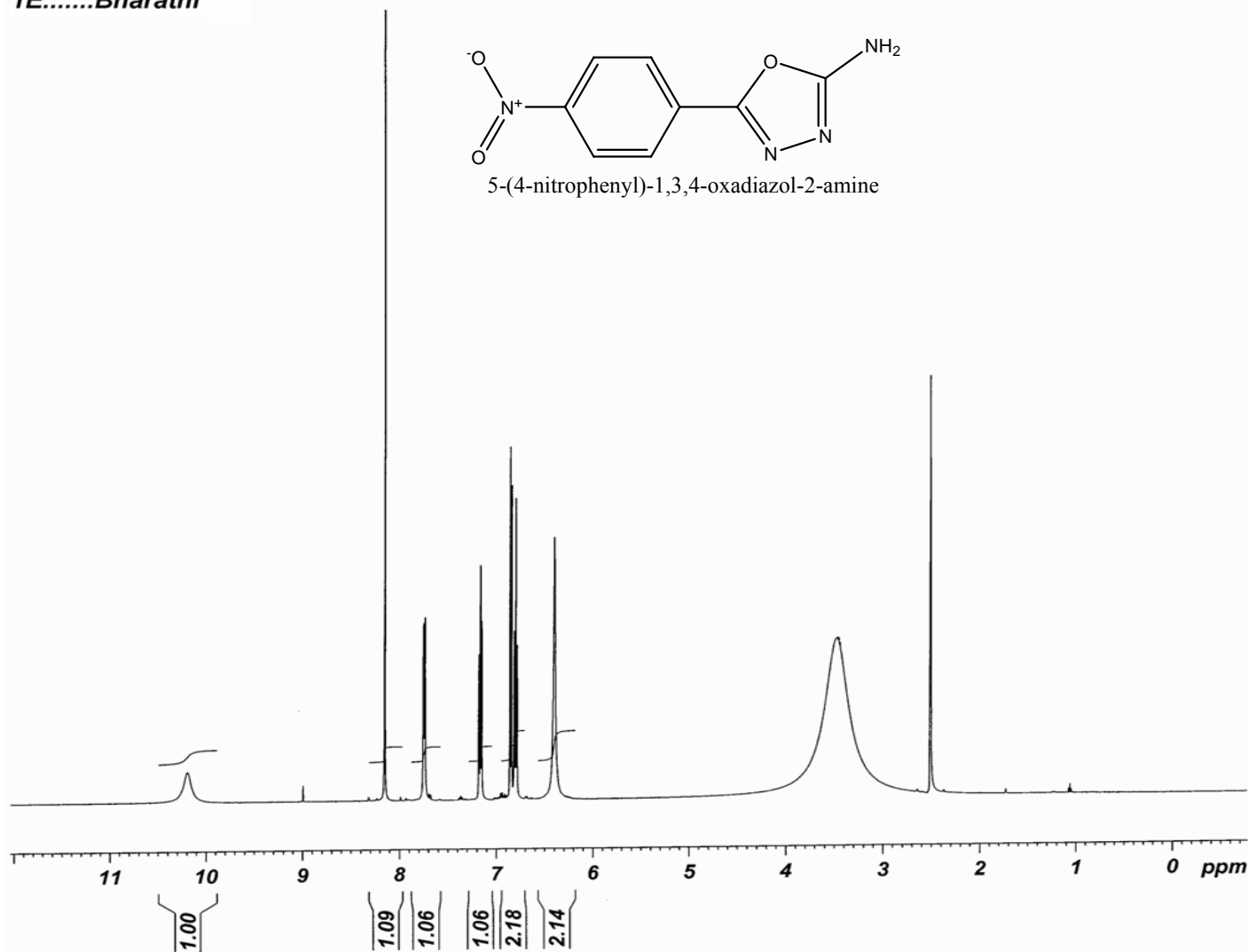
Fig. 19

1E.....Bharathi

SAIF, IIT Madras



5-(4-nitrophenyl)-1,3,4-oxadiazol-2-amine



Current Data Parameters  
NAME Dec31-2009  
EXPNO 6  
PROCNO 1

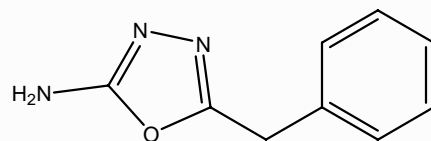
F2 - Acquisition Parameters  
Date\_ 20091231  
Time 16.30  
INSTRUM spect  
PROBHD 5 mm PABBO BB-  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.315264 Hz  
AQ 1.5860212 sec  
RG 90.5  
DW 48.400 usec  
DE 6.50 usec  
TE 297.8 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
NUC1 1H  
P1 10.65 usec  
PL1 0.00 dB  
PL1W 23.53637505 W  
SFO1 500.1330885 MHz

F2 - Processing parameters  
SI 32768  
SF 500.1300000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Fig. 20

1F.....Bharathi



5-Benzyl-1,3,4-oxadiazol-2-amine

SAIF, IIT Madras

Current Data Parameters  
NAME Dec31-2009  
EXPNO 2  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20091231  
Time 16.34  
INSTRUM spect  
PROBHD 5 mm PABBO BB-  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.315264 Hz  
AQ 1.5860212 sec  
RG 80.6  
DW 48.400 usec  
DE 6.50 usec  
TE 298.1 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
NUC1 1H  
P1 10.65 usec  
PL1 0.00 dB  
PL1W 23.53637505 W  
SFO1 500.1330885 MHz

F2 - Processing parameters  
SI 32768  
SF 500.1300000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

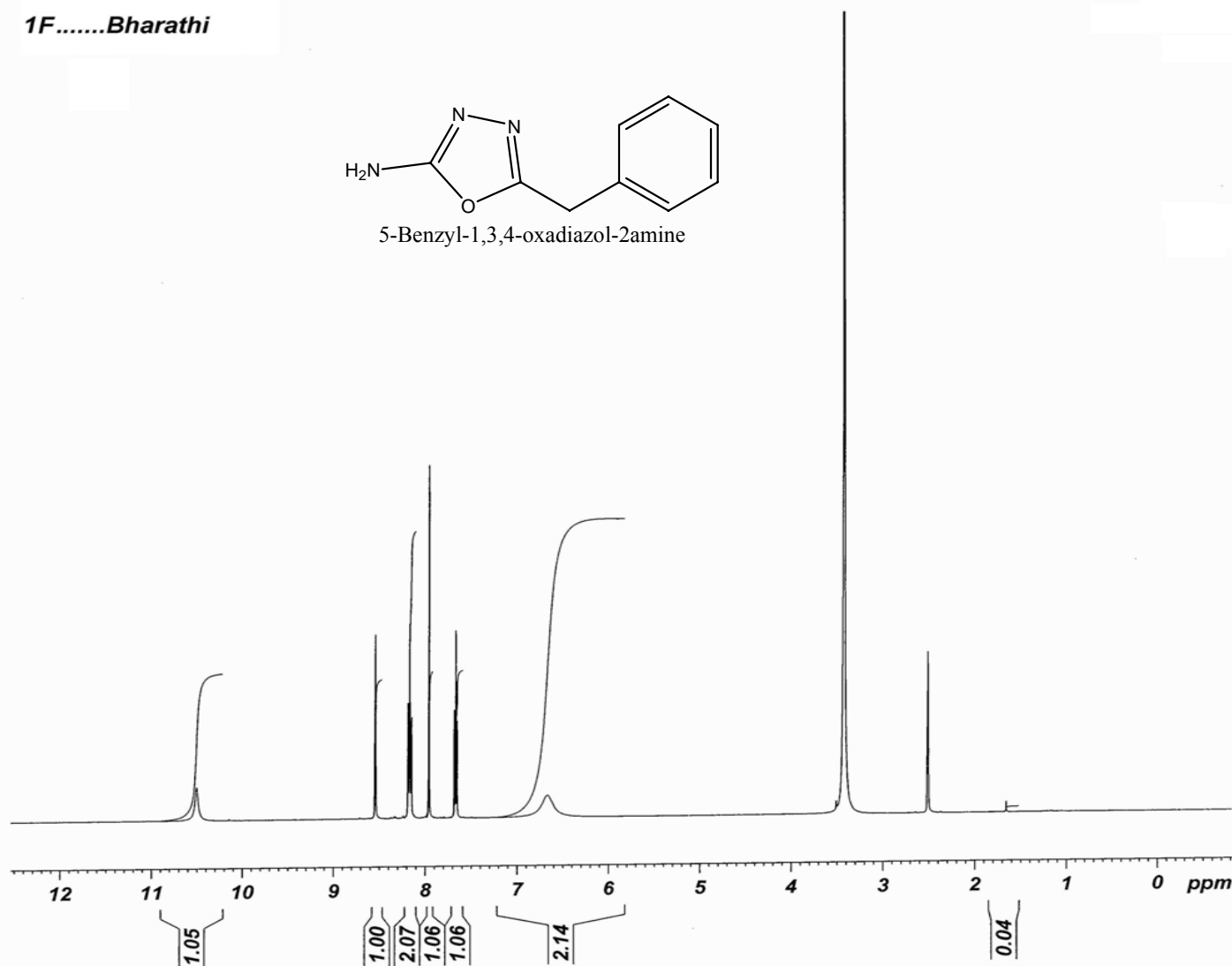
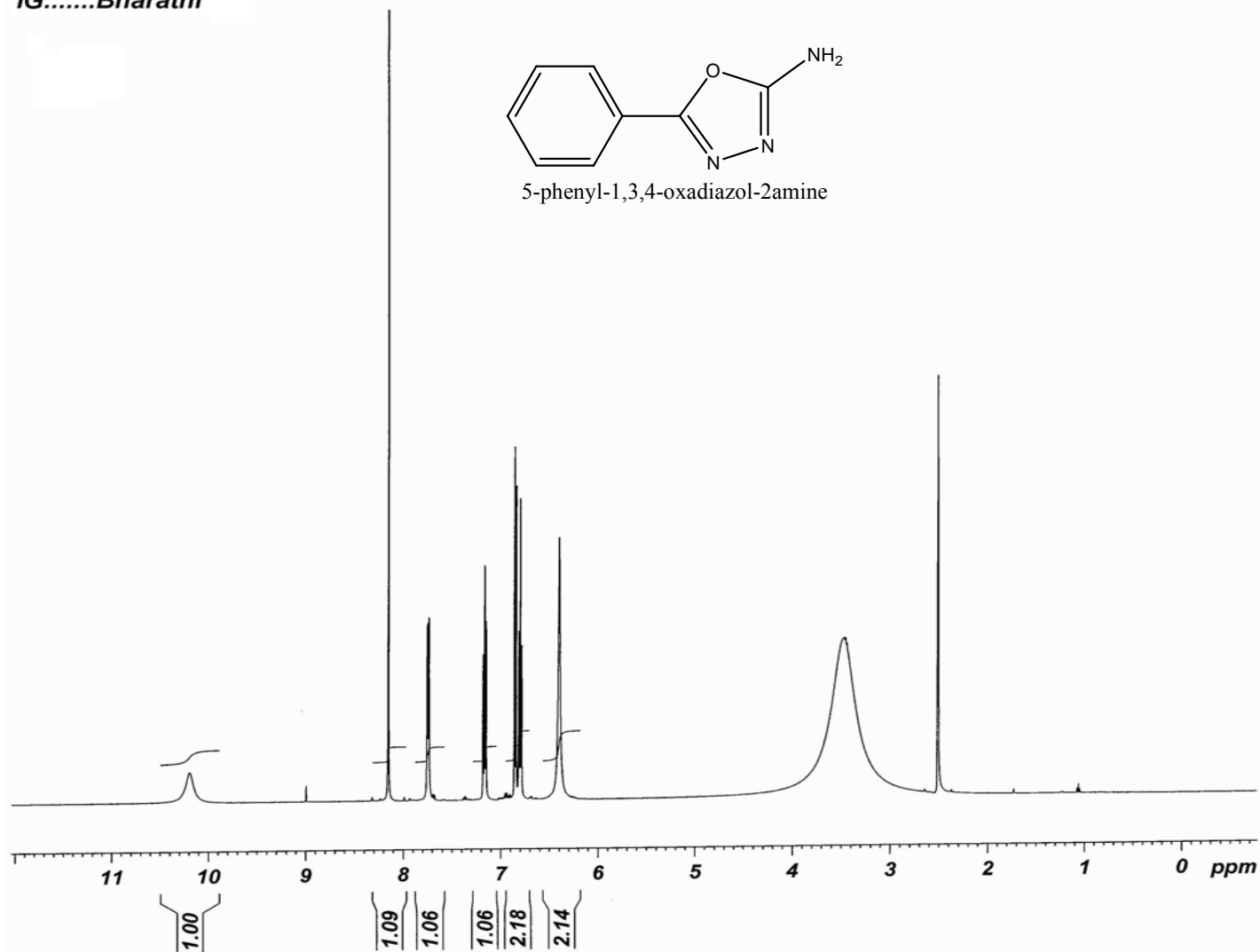
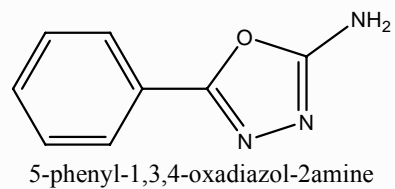


Fig. 21

1G.....Bharathi

SAIF, IIT Madras



Current Data Parameters  
NAME Dec31-2009  
EXPNO 6  
PROCNO 1

F2 - Acquisition Parameters  
Date\_ 20091231  
Time 16.38  
INSTRUM spect  
PROBHD 5 mm PABBO BB-  
PULPROG zg30  
TD 32768  
SOLVENT DMSO  
NS 16  
DS 2  
SWH 10330.578 Hz  
FIDRES 0.315264 Hz  
AQ 1.5860212 sec  
RG 90.5  
DW 48.400 usec  
DE 6.50 usec  
TE 297.8 K  
D1 1.00000000 sec  
TD0 1

===== CHANNEL f1 =====  
NUC1 1H  
P1 10.65 usec  
PL1 0.00 dB  
PL1W 23.53637505 W  
SFO1 500.1330885 MHz

F2 - Processing parameters  
SI 32768  
SF 500.1300000 MHz  
WDW EM  
SSB 0  
LB 0.30 Hz  
GB 0  
PC 1.00

Fig. 22

**Table X - *Invitro* Anti inflammatory Activity of Synthesized Compounds 1A-1G**

S.No.	Compounds	Concentration $\mu\text{g/ml}$ Absorbance at 540nm			% Prevention of Lysis
		25	50	100	
1	<b>1A</b>	0.15 $\pm$ 0.001**	0.19 $\pm$ 0.002*	0.20 $\pm$ 0.001*	69.6
2	<b>1B</b>	0.20 $\pm$ 0.04*	0.22 $\pm$ 0.07*	0.16 $\pm$ 0.04**	58.1
3	<b>1C</b>	0.35 $\pm$ 0.03*	0.36 $\pm$ 0.01*	0.38 $\pm$ 0.005*	28.6
4	<b>1D</b>	0.32 $\pm$ 0.03*	0.33 $\pm$ 0.04*	0.35 $\pm$ 0.05*	31.0
5	<b>1E</b>	0.22 $\pm$ 0.001*	0.28 $\pm$ 0.007*	0.27 $\pm$ 0.08**	60.5
6	<b>1F</b>	0.30 $\pm$ 0.04*	0.35 $\pm$ 0.04*	0.32 $\pm$ 0.04*	30.2
7	<b>1G</b>	0.20 $\pm$ 0.04*	0.27 $\pm$ 0.04*	0.25 $\pm$ 0.06*	43.3
8	<b>Control</b>	-	-	-	-
9	<b>Diclofenac</b>		0.14 $\pm$ 0.002*		74.8

Values are expressed as mean  $\pm$ SEM

P\* $<$ 0.01 – Moderate P\*\* $<$ 0.001 – Significant

One way ANOVA followed by Dunnett's 't' test

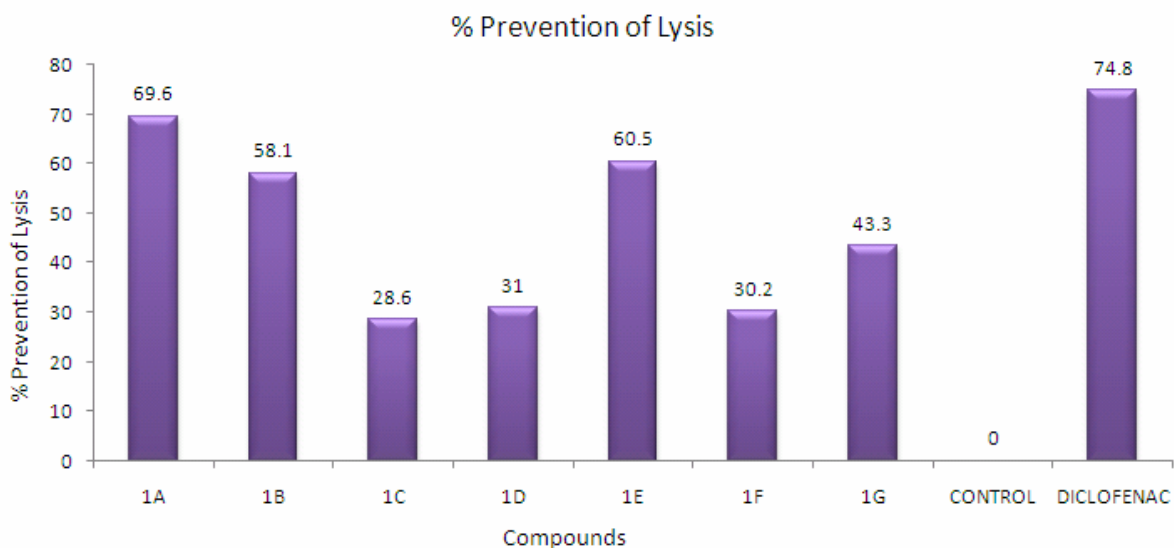
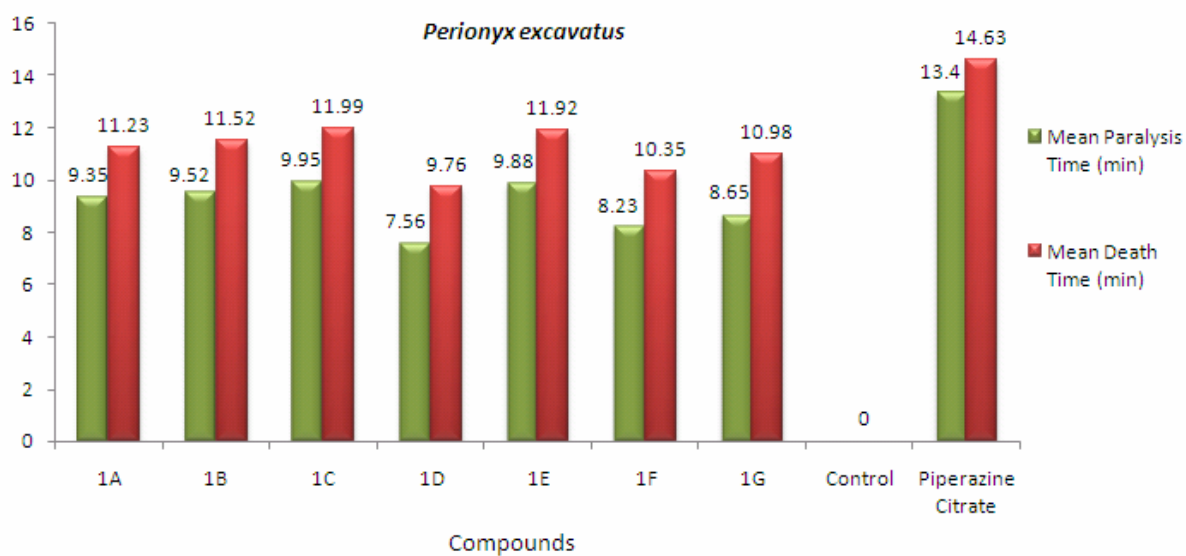
**Graph-1**

Table XI - Invitro Anthelmintic Activity of synthesized compounds 1A-1G

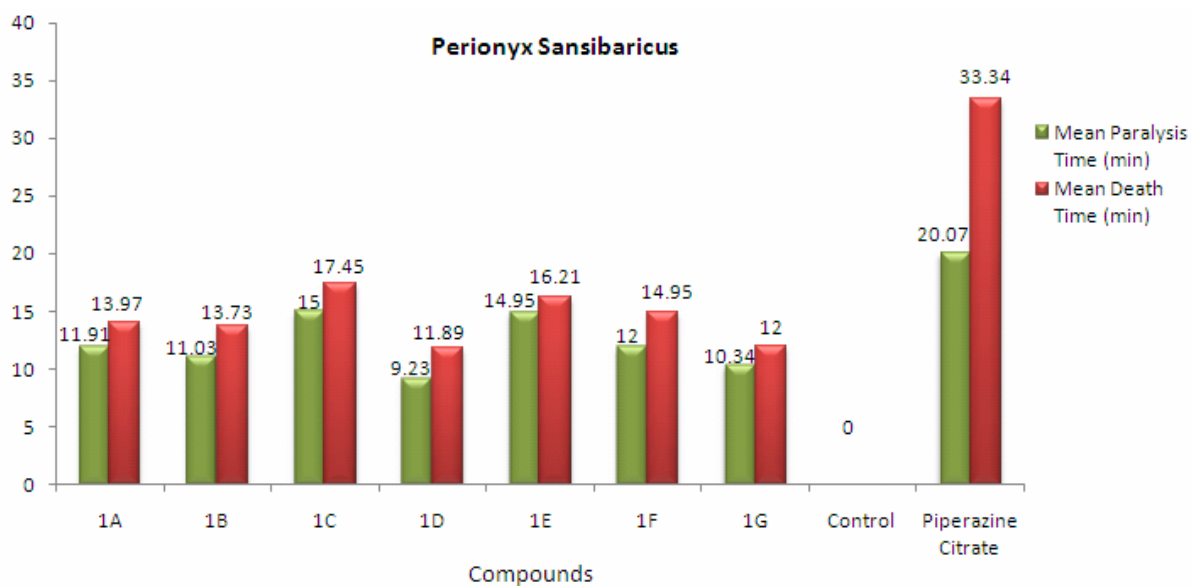
Compound	Earthworm Species			
	<i>P.excavatus</i>		<i>P.sansibaricus</i>	
	Mean Paralysis Time (min)	Mean Death Time (min)	Mean Paralysis Time (min)	Mean Death Time (min)
<b>1A</b>	09.35±0.67	11.23±0.53	11.91±0.99	13.97±0.64
<b>1B</b>	09.52±0.54	11.52±0.57	11.03±0.20	13.73±0.07
<b>1C</b>	09.95±0.51	11.99±0.98	15.00±0.62	17.45±0.60
<b>1D</b>	07.56±0.56	09.76±0.94	09.23±0.45	11.89±0.57
<b>1E</b>	09.88±0.90	11.92±0.99	14.95±0.63	16.21±0.96
<b>1F</b>	08.23±0.56	10.35±0.47	12.00±0.98	14.95±0.74
<b>1G</b>	08.65±0.23	10.98±0.34	10.34±0.47	12.00±0.95
<b>Control</b>	-	-	-	-
<b>Piperazine Citrate</b>	13.40±0.47	14.63±0.50	20.07±0.68	33.34±0.89

\* Data are given as the mean ±S.D





Graph-2



Graph-3

# *CHAPTER-VII*

## *SUMMARY & CONCLUSION*

## 7. SUMMARY AND CONCLUSION

Molecular modification of simple and complex chemical entities may lead to biological active compounds. Different types of approaches are made to derive such compounds which exhibit selective pharmacological activity. 1, 3, 4 oxadiazole derivatives show potent anti-inflammatory and anthelmintic activities.

This research work was oriented towards the finding of derivatives of 1, 3, 4 oxadiazole with anti inflammatory and anthelmintic activities. The different substituted aldehyde derivatives were synthesized by condensation reactions. Seven compounds have been synthesized and all the compounds were labeled 1A-1G.

Using different analytical techniques, namely, elemental analysis, IR, <sup>1</sup>HNMR and Mass spectroscopical studies the structure of the different substituted 1, 3, 4 oxadiazole derivatives were confirmed.

The newly synthesized 1, 3, 4 oxadiazole derivatives were evaluated for their *invitro* anti-inflammatory by membrane stabilization method. All the synthesized compounds showed anti inflammatory activity. Out of all the synthesized compounds 1A and 1B and 1E showed significant anti inflammatory activity. All the other compound shows moderate anti inflammatory activity when compared with that of standard diclofenac.

The literature shows that the compounds having methoxy, nitro, hydroxyl groups possess significant anti inflammatory activity<sup>33</sup>. The synthetic compounds 1A, 1B, 1E possess OCH<sub>3</sub>, OH, nitro group in its structure. So the anthelmintic activity may be due to the presence of the respective functional groups as evidence in literature.

The anthelmintic activity was carried out using two species of earth worm. The synthesized compounds show good anthelmintic activity out of all the synthesized compounds. Compound 1C and 1E showed good anthelmintic property. The activity may be due to the presence of methyl and nitro group in the compound which has been proved in earlier reports<sup>12</sup>.

# *CHAPTER-VIII*

## *BIBLIOGRAPHY*

## 8. BIBLIOGRAPHY

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# CHAPTER-IX

## APPENDICES

## 9. APPENDIX-I

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**APPENDIX- IV****List of Abbreviations**

mg	-	milligram
gm	-	gram
ml	-	milliliter
%	-	percentage
mm	-	millimeter
cm	-	centimeter
µg	-	microgram
m.p	-	melting point
pH	-	hydrogen ion concentration
<sup>1</sup> HNMR	-	Proton Nuclear magnetic Resonance
NMR	-	Nuclear magnetic Resonance
TMS	-	Tetra methyl silane
DMSO	-	Dimethyl sulfoxide
THF	-	Tetra hydro furan
HO	-	Hydroxyl ion
°C	-	Degree Centigrade
nm	-	nanometer
KBr	-	Potassium Bromide
SEM	-	Standard Error Mean
ANOVA	-	Analysis of Variance
O.D	-	Optical Density
COX	-	Cyclo Oxygenase Enzyme
NSAID	-	Non Steroidal Anti Inflammatory Drugs

PGG	-	Prostaglandins
UV	-	Ultra Violet
IR	-	Infra Red
HRBC	-	Human Red Blood Cell
FT	-	Fourier Tranformer
PPm	-	Parts Per Million
R <sub>f</sub>	-	Retardation Factor